H⁺ Fluxes in Excised Samanea Motor Tissue

I. PROMOTION BY LIGHT

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ABSTRACT

Previous investigators revealed that white light-promoted leaflet opening in Samanea saman (Jacq) Merrill depends upon K⁺ uptake by extensor cells and efflux from flexor cells of the pulvinus, while dark-promoted closure depends upon K⁺ fluxes in the opposite directions. We now monitored H⁺ fluxes during pulvinar movement to test a model proposing coupled H⁺/K⁺ fluxes. H⁺ fluxes were monitored by measuring changes in the pH of a weakly buffered solution (initial pH = 5.5) bathing excised strips of extensor or flexor tissue. White light at hour 3 of the usual dark period promoted pulvinar opening; H⁺ efflux from extensor cells and uptake by flexor cells, while darkness at hours 2 to 4 of the usual light period promoted pulvinar closure, H⁺ uptake by extensor cells and efflux from flexor cells. The following conditions altered H⁺ fluxes during dark-promoted closure. (a) Light reversed the directions of the fluxes in both extensor and flexor cells. (b) Anoxia increased the rate of H⁺ uptake by extensor cells and promoted H⁺ uptake (rather than efflux) by flexor cells, consistent with an outwardly directed H⁺ pump. KCN showed similar effects initially, but they were transient. (c) Increase in external pH from 5.5 to 6.7 promoted H⁺ efflux (rather than uptake) by extensor cells and increased the rate of H⁺ efflux from flexor cells, presumably by decreasing the rate of inward diffusion. (d) Change in external K⁺ did not alter H⁺ fluxes by extensor cells, but removal of external K⁺ decreased the rate of H⁺ efflux from flexor cells by 70%. These observations support a model for coupled H⁺/K⁺ fluxes in pulvinar cells during light- and dark-promoted leaflet movements.

Leaflet movements in nyctinastic plants are turgor regulated and dependent upon redistribution of ions, mainly K⁺, among cells in different regions of the pulvinus, the motor organ at the base of the leaflet (reviewed in 20). The uptake of K⁺ is coupled to the efflux of H⁺ in many plant tissues (1, 15, 16, 26). In stomatal guard cells, for instance, light-induced increase in turgor is accompanied by K⁺ influx, H⁺ efflux (18) and rapid hyperpolarization (13), suggesting that H⁺ ions are secreted by an electrogenic pump (13). Previous investigators (9, 21) have postulated that K⁺ fluxes into and out of pulvinar motor cells might also be coupled to oppositely directed H⁺ fluxes. Figure 1 shows the well-documented (23) directions of K⁺ fluxes as cells change in turgor, and the postulated model (21) for coupled H⁺ fluxes; the size of the cell indicates the state of turgor.

H⁺ fluxes through membranes of other plant cells have been monitored by measuring changes in the pH of a solution bathing excised groups of homogeneous cells (1, 3, 5, 8, 14, 19). Inasmuch as the pulvinus is divided into two functionally different halves

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FIG. 1. A model proposing that K⁺ fluxes into and out of swelling and shrinking pulvinar motor cells are coupled to H⁺ fluxes. (Other solutes involved in turgor regulation (9, 21) are not considered in this model.) → H⁺, H⁺ pump (the larger arrow indicates higher activity); H⁺ ↔ H⁻ leak (the larger arrow indicates greater leakiness); K⁺, net K⁺ flux, with direction opposite to that of net H⁺ flux. Net H⁺ flux is outward when pump activity is high and/or permeability to H⁺ is low; net H⁺ flux is inward when pump activity is low and/or permeability to H⁺ is high. (extensor and flexor), it is necessary to separate these regions before measuring H⁺ fluxes. The precise boundary between extensor and flexor in the Samanea pulvinus was established recently (22), facilitating the separation of these two cell types. We now report on our study of H⁺ fluxes into and out of extensor and flexor pulvinar tissue from Samanea. We studied H⁺ fluxes during both light-promoted leaflet (pulvinar) opening and dark-promoted closure, examining dark-promoted responses in the greatest detail because they are faster and larger than light-promoted responses.

MATERIALS AND METHODS

Plant Material. Samanea saman (Jacq) Merrill plants were grown from seed in controlled chambers with 16-h light/8-h dark cycles at 25.5 ± 1.5°C and 70% RH. Light was provided by cool-white fluorescent lamps at 240 μmol m⁻² s⁻¹. Uniform plants of similar age were selected. Terminal secondary pulvinii from the third to the tenth uppermost mature leaves were harvested at hours 2 to 7 of the dark period for experiments testing light-promoted opening, and at hours 2 to 4 of the light period for experiments testing dark-promoted closing. Pair pulvinii from the same leaves were used for comparative treatments.

Angle Measurements. To test the effect of various treatments on pulvinar angle, a terminal or subterminal pulvinus was excised attached to a 2-cm section of rachilla (23). In experiments that test the effect of pulvinar submersin (Fig. 3), the pulvinus and attached rachilla were placed horizontally on the bottom of a Petri dish.

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3 Extensor cells swell during opening and shrink during closure, while flexor cells shrink during opening and swell during closure.
dish, secured in place with plasticine on the rachilla, and covered with solution. O₂ was bubbled through the bathing solution when indicated. In all other experiments, the rachilla was vertical with only the terminal end in solution. The angle between the pulvinus and the rachilla was measured with a protractor. Each point in the Figures represents the average angle of 5 to 12 pulvini from one of three replicate experiments; error bars in Figure 3 represent SD. SD (not shown) for points in Figures 4 and 5 are of the same magnitude.

Measurement of H⁺ Fluxes. H⁺ fluxes were determined by changes in the pH of a solution bathing excised extensor or flexor tissue. Strips of tissue were excised with a sharp razor immediately after the pulvinus had been cut from the plant (Fig. 2). Only outer cortical cells were included in the strips. Although this procedure minimizes variability, it does not assure that all cells within a strip are uniform, since K⁺ distribution varies for different cells within the extensor and flexor outer cortices (20).

Extensor or flexor tissue from 12 pulvini was used for each experiment. The total dry weight averaged 11 mg (extensor) and 15 mg (flexor). The experiment described in Figure 7 used tissue from the rachis (dry weight was 14 mg). The tissue was washed for 10 to 20 min in a fresh oxygenated ‘washing solution’ of the same composition as the solution used for pH measurements (described below). When KCN was being tested, it was added only to the solution used to measure pH changes. After the tissue had been washed, it was transferred to a 2-ml beaker containing 0.5 ml of a fresh solution. O₂ was bubbled continuously, except when the effect of anoxia was being tested; in this case N₂ replaced O₂ and the system was enclosed in a chamber with a N₂ atmosphere. Bubbling O₂ (or N₂) stirred the tissue continuously, thereby assuring that the pH of the solution was uniform at all times.

The solution pH was monitored with a micro combination pH probe (model MI-410; Microelectrodes Inc., Londonderry, NH) connected to a pH meter (model 407A/L; Orion Research Inc., Cambridge, MA), and the voltage signal was recorded on a twopen chart recorder (model B5217-51; Houston Instruments, Austin, TX). Inasmuch as we had two replicate set-ups, we could monitor two different treatments simultaneously. Figures 4 to 9 show typical recordings, redrawn for appropriate scale. Data in Table II were taken from continuous recordings. Data in Table I were obtained by measuring pH at 15-min intervals, thereby permitting comparison of eight different treatments. Each value in the Tables represents the mean of three different experiments ± SD. Each experiment was performed at least three times with similar results. Occasional aberrant results probably resulting from errors in the excision and preparation of the tissue, or from nonuniform plant material, were discarded.

Illumination and Temperature. When white light was required during an experiment, a Westinghouse 75-w incandescent flood lamp was used as the source (280 μmol m⁻² s⁻¹ in the 400–700 nm range). It did not alter the response of the electrode or recorder (Fig. 7). A safelight (two Sylvania 15-w green fluorescent tubes covered with two layers of Roscolene celluloid No. 874, Roscoe Labs, Portchester, NY) provided illumination during dark conditions. The temperature was 30°C during all experiments.

Solutions. All solutions contained 0.1 mm Mes buffer, 1 mm Ca(NO₃)₂, and 1 mm MgSO₄. Most of the solutions also contained 5 mm K₂SO₄, with pH adjusted to 5.5 with NaOH except where otherwise indicated. O₂ was bubbled through solutions during pH adjustment. For experiments that test the influence of external [K⁺] (Table I), KCl replaced K₂SO₄. FC⁺ (Fig. 7) was generously supplied by Dr. E. Marré. For experiments with KCN (Fig. 8), the pH was adjusted to 5.5 with HCl (the final Cl⁻ concentration was 0.2 mm). Loss of KCN due to volatilization was minimized by covering the vial with paraffin. Consequently, the solution was in contact with a HCN-enriched atmosphere.

Titration Curves. Inasmuch as the bathing solutions were buffered, H⁺ fluxes would be underestimated if calculated directly from the initial and final pH measurements. We therefore titrated the experimental solution with HCl or NaOH, to evaluate the relationship between equivalents of H⁺ or OH⁻ added and the solution pH.

RESULTS

Samanea pulvinus move slowly in response to a circadian oscillator, and more rapidly in response to light perturbations. We measured H⁺ fluxes during light-promoted opening and dark-promoted closure, because these movements are faster and can be manipulated more easily than circadian movements.

Because our method for monitoring H⁺ fluxes involves submerging pulvinar tissue in a liquid medium, it was important to determine the consequences of submersion on pulvinar movements. Figure 3 shows that excised, submerged pulvinus opened in the light and closed in the dark at rates similar to those of excised, upright, nonsubmerged controls if O₂ was bubbled through the medium, confirming results of Sweet and Hillman (27). Therefore, except where noted, O₂ was bubbled through the bathing solution continuously. Nevertheless, a pulvinus submerged for more than 3 h with or without O₂, invariably started to close. Subsequent experiments with submerged tissue were completed within 1 h.

H⁺ Fluxes during Pulvinar Opening. At hour 3 of the dark period, pulvinus are in the closed position, but they open when excised and illuminated with white light (Fig. 4A). Extensor and flexor tissues were excised at this time, prepared for pH measurements, and the effect of white light on the pH of the bathing solution was then monitored (Fig. 4B). Extensor tissue acidified

Table I. Effect of External K⁺ on Dark-Promoted H⁺ Fluxes from Extensor and Flexor Tissue into the Bathing Solution

<table>
<thead>
<tr>
<th>External KCl</th>
<th>ΔpH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Extensor</td>
</tr>
<tr>
<td>mm</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>+0.31 ± 0.02</td>
</tr>
<tr>
<td>5</td>
<td>+0.25 ± 0.03</td>
</tr>
<tr>
<td>10</td>
<td>+0.24 ± 0.05</td>
</tr>
<tr>
<td>50</td>
<td>+0.31 ± 0.09</td>
</tr>
</tbody>
</table>

Abbreviation: FC, fusicoccin.

![Fig. 2. Schematic drawing of the Samanea pulvinus. (a), Outer cortex; (b), inner cortex; (c), vascular tissue and collenchyma; (d), boundary between extensor and flexor cells (22). The darkened areas indicate the strips of tissue excised for pH measurements.](https://www.plantphysiol.org)
Table II. Effect of External pH on Dark-Promoted H⁺ Fluxes from Extensor and Flexor Tissue into the Bathing Solution

Tissue was excised at hour 4 of the usual light period, washed in the growth chamber under white light, and then darkened. Values represent changes in the pH of the solution (mean ± SD) during the first 45 min of darkness and H⁺ fluxes, calculated with aid of a titration curve. Positive values indicate net H⁺ uptake from the medium, while negative values indicate net H⁺ efflux.

<table>
<thead>
<tr>
<th>Initial pH</th>
<th>Extensor</th>
<th>Flexor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ΔpH</td>
<td>ΔH⁺</td>
</tr>
<tr>
<td>5.50</td>
<td>+0.23 ± 0.10</td>
<td>+12.5</td>
</tr>
<tr>
<td>6.70</td>
<td>−0.83 ± 0.23</td>
<td>−43.0</td>
</tr>
</tbody>
</table>

Fig. 3. Effect of submersion on pulvinar opening and closure, with and without supplemental O₂. A, Opening promoted by white light at hour 3 of the usual dark period. B, Closure promoted by darkness at hour 4 of the usual light period. Upright control (○); submerged horizontal pulvinus + O₂ (○) or − O₂ (△).

The medium about 0.2 pH unit during 60 min while flexor tissue alkalized the medium about 0.4 unit during the same interval. Stable external pH was not achieved during this period. It is unlikely that the release of vacuolar contents from broken cells influenced the pH changes, since doubling the time the tissue was washed before illumination did not alter our results.

The response to illumination was also tested at other times during the dark period. Flexor cells alkalized the medium in response to light presented between hours 2 and 6 of the usual dark period; a maximum response was obtained at hour 3. Extensor cells acidified the medium when light was presented between hours 2 and 3 of the usual dark period; however, they did not change the pH of the solution or make it slightly more alkaline (about 0.1 pH unit/60 min) if light was presented later in the dark period. The difference in response at different times is probably a consequence of the state of the internal oscillator which controls the sensitivity of the pulvinar movement to light (21), and might also control sensitivity of pulvinar cells to injury and submersion.

H⁺ Fluxes during Pulvinar Closure. When *Samanea* are exposed to 16-h light/8-h dark cycles, pulvini open completely during the first 60 to 90 min of light, and then close partially if darkened during the next few hours (27). Changes in the pH of the solution bathing excised extensor and flexor tissue accompany dark-promoted closure (Fig. 5, A and B). Extensor tissue alkalized the medium about 0.3 pH unit/45 min, while flexor tissue acidified the medium about 0.4 pH units during the same interval. Both these pH changes are opposite in direction to the ones recorded during...
LIGHT-PROMOTED $H^+$ FLUXES IN SAMANEA

Fig. 6. Effect of light-dark transitions on the pH of a solution bathing (A) extensor and (B) flexor tissue. The tissue was excised at hour 2 of the usual light period, washed in the light, and then darkened for the entire monitoring period (control) or subjected to changes in illumination as indicated. (l), Lights on; (f), lights off.

Fig. 7. Effect of light-dark transitions on the pH of a solution bathing rachis tissue. The tissue was excised at hour 4 of the usual light period, washed in the light, and then subjected to changes in illumination as indicated. The concentration of FC was 10 μM. (l), Lights on; (f), lights off.

during light-promoted opening (Fig. 4B).

To determine whether darkness is necessary for these large pH changes, we tested the effect of light-dark and dark-light transitions, initiating the experiment at hour 2 of the light period (rather than hour 4, as in Fig. 5). The pulvinus always closes 10° or more following excision and submersion (27 and our unpublished observations), but this response is minimized at hour 2, permitting the effect of the transition from white light to darkness to be most fully expressed. The pH of the bathing solutions remained relatively constant while the tissue was in white light rather than darkness (Fig. 6) except for a rapid small alkalinization of the solution bathing extensor tissue. Furthermore, the direction of dark-promoted pH changes could be reversed by white light. The lag time between light-dark or dark-light transitions and detectable pH response varied from 3 to 8 min for different experiments.

To determine whether extracellular pH changes were also produced by cells in the cortex of the rachis, tissue outside the central vascular core of the rachis was excised, washed, and then incubated in a bathing solution of the same composition used for experiments with pulvinar tissue. Rachis tissue slightly acidified the medium (Fig. 7); light-dark cycles did not alter the rate of acidification within the sensitivity of our detection system. If 10 μM FC was added to the bathing solution, the rate of acidification was 3 times as high as that of the control, indicating that the tissue was physiologically active. Thus, large light-promoted pH changes do not occur in all tissues and may be confined to the pulvinus, as are light-promoted changes in cell turgor.

Effect of Cyanide. It was important to determine whether light-promoted $H^+$ fluxes have an energy-requiring component. We used cyanide rather than some other inhibitor to block ATP synthesis, since HCN has a high pKₐ (≈ 9); consequently, HCN (the undissociated form of the acid) is the predominant form at the pH range of the bathing solution. Therefore, this inhibitor should not alter the buffering capacity of the external medium, and HCN should diffuse passively into the cells.

Excised pulvini incubated in 1 mM KCN closed in the dark at a rate similar to that of controls (Fig. 5A). Nevertheless, 1 mM KCN had large effects on pH changes (Fig. 8), initially increasing medium alkalinization by extensor tissue and causing flexor tissue to alkalinize the medium instead of acidifying it. However, significant reversions of the initial effects were observed after 10 to 15 min for both extensor and flexor tissue. The time course and extent of the reversions were variable, but after 45 min the external pH values were similar to those of controls. KCN at 0.5 mM had no appreciable effect on pH changes of the medium while 2 mM had the same effect as 1 mM, within the resolution of our system.

Effect of O₂ Depletion. Since plants contain a CN-insensitive respiratory pathway (10), it was important to test the effect of inhibiting both CN-insensitive and CN-sensitive respiration simultaneously. Depletion of O₂ serves this purpose and has some advantages over chemical inhibitors of the alternative oxidase (e.g. hydroxamic acids), since depletion of O₂ does not alter...
flexor tissue. In the extensor, net $H^+$ uptake was observed when the initial pH of the medium was 5.5, but net $H^+$ efflux was observed when the initial pH was 6.7. In the flexor, net $H^+$ efflux was observed whether the initial pH was 5.5 or 6.7, but the rate of $H^+$ efflux was much greater at the higher pH. Note that the difference in proton flux between flexor and extensor tissue is 27.5 neq/45 min at pH$_5$ = 5.5 and 24.5 neq/45 min at pH$_6$ = 6.7. Thus, flexor cells should take up more $K^+$ than extensor cells at both pH$_4$ values. Pulvinii closed at the same rate (40°/45 min) at both pH$_4$ values, consistent with the above prediction.

**DISCUSSION**

Relationship between pH Changes and $H^+$ Fluxes. Time-dependent changes in pH of the bathing solution have been interpreted by others (1, 3, 5, 8, 14, 19) to indicate $H^+$ fluxes into and out of cells in submerged tissue. Nevertheless, several possible artifacts can affect the accuracy of this conversion. (a) Broken cells might release vacuolar contents to the medium. The pH of cell vacuoles is usually less than 5.5 (11); therefore, vacuolar sap would be expected to acidify the bathing solution. (b) Protons might be released from wall sites by cations in the bathing solution; if so, they would acidify the medium. (c) Weak organic acids (e.g., malic) might be released from the tissue during the monitoring period, thereby increasing the buffering capacity of the bathing solution. (d) Variations in the amount of CO$_2$ (released to the medium during respiration and taken up during photosynthesis) would contribute toward variation in pH.

It is unlikely that the release of vacuolar sap had a significant effect in our system, since doubling the time the tissue was washed did not change the magnitude of the pH changes. It is also unlikely that cation exchange in wall sites affected our data significantly, since this process would not be expected to require metabolic energy, yet inhibition of ATP synthesis prevented medium acidification (Figs. 8 and 9). If weak organic acids were released, our conversion of pH to $H^+$ equivalents (Table II) would underestimate rather than overestimate $H^+$ fluxes. It is unlikely that CO$_2$ had a substantial effect on our results, inasmuch as it was continuously displaced by O$_2$ bubbled through the medium. Furthermore, extensor and flexor tissue respond oppositely to light-dark transitions (Figs. 4 and 5), while pH of the medium surrounding the tissue was insensitive to light (Fig. 7). We therefore conclude that acidification of the medium indicates net $H^+$ flux out of pulvinar cells, and alkalization of the medium indicates net $H^+$ flux into pulvinar cells, while the magnitude of the fluxes may be underestimated.

The relationship between the fluxes we observed and those occurring in vivo is more difficult to assess. Although submerging a whole pulvinus does not alter its movements if O$_2$ is bubbled through the solution, we do not know how submergence affects $H^+$ fluxes in excised bits of tissue. Furthermore, injury promotes $H^+$ uptake by cells of other plants (2) and might have contributed to the fluxes we observed, thereby partially masking the effects of an outwardly directed $H^+$ pump. Nevertheless, it is unlikely that submergence and injury are the major contributors to the fluxes we detected, since: (a) $H^+$ fluxes occur in opposite directions in extensor and flexor tissue; (b) they are light-dark reversible (Fig. 6); and (c) they are altered by blockage of metabolic energy (Figs. 8 and 9).

**Data Supporting the Model for Coupled $K^+/H^+$ Fluxes.** Leaflet opening in *Samaenea* (23) and other nyctinastic plants (reviewed in 20) is dependent upon $K^+$ uptake by extensor cells and efflux from flexor cells, while leaflet closure is dependent upon $K^+$ efflux from extensor cells and uptake by flexor cells. The $H^+$ fluxes we monitored during leaflet opening (Fig. 4) and closure (Fig. 5) are opposite in direction to those reported for $K^+$ (23), consistent with the model for coupled $H^+/K^+$ fluxes (Fig. 1) initially proposed in Satter and Galston (21). The model (21) postulates that: (a) net buffering capacity, pH, or anion composition of the medium.

Excised pulvinii darkened in the absence of O$_2$ did not close, confirming results of Sweet and Hillman (27). Anoxia increased medium alkalinization by extensor tissue and caused medium alkalinization rather than acidification by flexor tissue (Fig. 9, A and B). These differential responses were detectable at the beginning of the dark period and persisted throughout the monitoring period.

**Effect of External [K$^+$].** Determining the effect of external [K$^+$] on medium acidification and alkalinization is important for understanding the relationship between K$^+$ and $H^+$ fluxes. External K$^+$ was increased from 0 to 50 mm, using KCl rather than K$_2$SO$_4$. Since Cl$^-$ is highly concentrated in *Samaenea* pulvinar tissue (20), it would not be expected to have a toxic effect when present in the medium at high concentration. During dark-induced closure, change in external [K$^+$] did not have a significant effect on net $H^+$ uptake by extensor tissue (Table I). However, solutions that lacked K$^+$ reduced net $H^+$ efflux from flexor tissue to less than half of the value obtained in the presence of 5 mm K$^+$. These differences were apparent from the beginning of the monitoring period to its termination. The promotive effect of external K$^+$ on $H^+$ efflux from flexor cells, saturated at 5 mm.

**Effect of External pH.** $H^+$ fluxes in some tissues are regulated in part by external pH (15, 19). We therefore compared dark-promoted changes in the pH of the bathing solution when the initial pH was 5.5 to those obtained when the initial pH was 6.7 (Table II); changes in pH were then converted to $H^+$ fluxes. Errors that could affect this conversion are evaluated in "Discussion".

External pH has large effects on $H^+$ fluxes in both extensor and flexor tissue. The tissue was excised at hour 4 of the usual light period, washed in the growth chamber under white light, and then darkened. (N$_2$). Vials containing the tissue were in a nitrogen atmosphere and nitrogen was bubbled through the solution. (O$_2$), Vials containing the tissue were in air and O$_2$ was bubbled through the solution.

**Fig. 9.** Effect of anoxia on dark-promoted changes in the pH of a solution bathing (A) extensor or (B) flexor tissue. The tissue was excised at hour 4 of the usual light period, washed in the growth chamber under white light, and then darkened. (N$_2$). Vials containing the tissue were in a nitrogen atmosphere and nitrogen was bubbled through the solution. (O$_2$), Vials containing the tissue were in air and O$_2$ was bubbled through the solution.

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H⁺ flux depends upon the dynamic equilibrium between energy-dependent H⁺ secretion and inward diffusion; (b) this equilibrium changes rhythmically during the circadian cycle; (c) the equilibrium can also be altered by light. Postulate 'b' is analyzed in a separate publication (7), while 'c' is validated by data in Figure 6. Postulate 'a' is supported by the following data.

(a) A large alkalinization of the solution bathing both extensor and flexor tissue was observed in the presence of KCN (Fig. 8) or in the absence of O₂ (Fig. 9). Both of these treatments promoted net H⁺ uptake by both extensor and flexor tissue, as would be expected if outward pump activity were decreased by reduction in ATP synthesis, while the inward leak was unaffected. Although effects of KCN were transient, membrane potential displays similar kinetics in other CN-treated plants (12). Recovery of CN-treated tissue has been interpreted as due to activation of an alternative oxidase (i.e. cyanide-resistant respiration; 10).

(b) Increase in external pH from 5.5 to 6.7 (Table II) increased the magnitude of H⁺ efflux from flexor cells and promoted net H⁺ efflux rather than uptake by extensor cells. The model predicts such results, since increase in external pH would reduce the pH gradient across the plasma membrane, thereby decreasing the rate of inward diffusion, and possibly also increasing the activity of the outward pump (12, 15, 25). We considered whether the magnitude of net H⁺ efflux from extensor and flexor tissue at pHₐ = 6.7 might be overestimated, due to H⁺ released by the dissociation of carbonic acid (pKₐ = 6.3). However, other investigators who studied this effect in detail (2, 16) concluded that CO₂ effects are minor when O₂ is bubbled through solutions continuously, as in our experiments.

(c) During dark-induced closure, variation in external K⁺ from 0 to 50 mM did not affect the rate of H⁺ uptake by extensor cells (Table I), consistent with the model, since extensor cells lose K⁺ to the medium during dark-promoted closure. Much higher K⁺, by contrast, might be expected to interfere with H⁺ uptake; 500 mM K⁺ severely inhibited dark-promoted closure in the related species Albizia julibrissin, although 200 mM K⁺ was ineffective (24). Inclusion of a low level of K⁺ in the medium was, however, required for maximum rates of H⁺ efflux from flexor cells, as would be expected, since the model predicts that K⁺ moves inward in response to the negative internal potential generated by H⁺ secretion. In the absence of adequate external K⁺, the internal potential would rapidly increase (become more negative), in turn reducing H⁺ pump activity (25).

Data in Table I are also consistent with a modification of our model to include chemical coupling between H⁺ and K⁺. If the coupling is chemical, K⁺ would move inward and H⁺ outward through the same transport structure; thus, adequate external K⁺ as well as internal H⁺ would be required for maximum pump activity. Although further studies are required to discriminate between these two possibilities, we have shown, as have other investigators (1), that the rate of H⁺ efflux from excised tissue depends upon the [K⁺] of the medium.

Thus, the general model of coupled H⁺ efflux/K⁺ uptake in plant cells (1, 16, 26) applies to pulvini, although the mechanism for movement of CI⁻ (20), Ca²⁺ (Satter and Garber, unpublished data), and other ions essential for pulvinar function (9, 21) remains to be determined.

Relationship of This Study to Previous Work. A previous investigation (17) demonstrated rapid hyperpolarization of Samanea flexor cells during dark-induced leaflet closure, although the ion(s) responsible for the potential changes were not determined. Our data suggest that electrogenic H⁺ secretion may be involved, although the possibility of rapid changes in K⁺ permeability cannot be excluded. Because the changes in membrane potential (17) occur much faster than the resolution time of our system, it will be interesting to investigate H⁺ fluxes by a method with better temporal resolution (e.g. insertion of a microelectrode in the cell wall).

Light-induced changes in membrane potential (6, 13, 25) and H⁺ fluxes (5, 8, 14, 18) have also been reported in other systems. Our system offers the interesting possibility of studying fluxes that occur in opposite directions in different parts of the tissue. Furthermore, the activity of the H⁺ pump and/or the magnitude of the leak vary during the daily cycle (7), providing an unusual opportunity for testing biological clock models based on trans-membrane H⁺ gradients (4).

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