

Communication

Effects of Temperature on H⁺ Uptake and Release During Circadian Rhythmic Movements of Excised *Samanea* Motor Organs¹

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ABSTRACT

A previous study revealed that *Samanea saman* leaflets open more completely and close less completely as temperature is increased. We now demonstrate that, as temperature is increased, extensor cells release more H⁺ during their swelling phase (opening), but flexor motor cells release less H⁺ during their swelling phase (closure).

Leaflets of *Samanea saman* open and close with a circadian rhythm during a prolonged dark period. These movements are due to the rhythmic expansion and shrinkage of cortical cells in the motor organ (pulvinus), in turn dependent upon circadian rhythms in ion fluxes (mainly K⁺ and Cl⁻) accompanied by water fluxes. Cells in the extensor region of the pulvinus take up K⁺, Cl⁻, and water during leaflet opening and lose both ions and water during closure, while cells in the opposing (flexor) region behave in the reverse manner (7).

Rhythmic leaflet opening is promoted by an increase in temperature, while rhythmic leaflet closure is promoted by a decrease in temperature (7). These effects were originally interpreted in terms of a 'tension/relaxation' hypothesis, *i.e.* the 'open' phase requires metabolic energy generation but the 'closed' phase does not (5-7). However, it is difficult to reconcile this interpretation with data indicating that K⁺ and Cl⁻ accumulate in flexor cells during closure (7), since the accumulation of K⁺ and Cl⁻ utilizes metabolic energy, directly or indirectly. Even if K⁺ enters motor cells passively, for example by diffusion through a channel in the plasma membrane, energy would be required to establish an electrochemical gradient that favors inward diffusion.

We recently reported rhythmic changes in H⁺ fluxes from excised motor tissue (3) and suggested that rhythmic changes in the proton-motive force established by the H⁺ pump may energize rhythms in the uptake of K⁺ and Cl⁻. This 'chemiosmotic' hypothesis predicts that the effect of temperature on leaflet movement is mediated by the effect of temperature on the proton-motive force. It acknowledges that metabolic energy is utilized during both opening and closure, in contrast to predictions of a tension/relaxation hypothesis.

We now describe effects of temperature on H⁺ fluxes in excised extensor and flexor tissue during rhythmic leaflet opening

and closure. Our data are consistent with a chemiosmotic explanation for rhythmic leaflet movement.

MATERIALS AND METHODS

Plant Material and Experimental Conditions. *Samanea saman* plants were grown with 16 h photoperiods (200 μE m⁻² s⁻¹) at 26°C ± 1.5°C. Pulvini were cut under dim green 'safelight' at h 4 of DD², which started at the beginning of the usual dark period. They were supplied with 50 mM sucrose and kept in DD for the remainder of the experimental period, as described in Lee and Satter (3).

Measurements of pH Changes and H⁺ Flux. At h 5 or 18 of DD, strips of extensor or flexor tissue were excised from 10 pulvini (average dry weight per 10 pulvinar strips = 6.3 mg extensor and 11 mg flexor). Regions of the pulvinus utilized are shown in Figure 2 of Iglesias and Satter (2). The excised strips of tissue were rinsed briefly and submerged in 500 μl of a bathing solution maintained at 18, 22, 26, or 30°C ± 0.5°C. The solution contained 0.1 mM MES (pH adjusted to 5.5 with NaOH or HCl), 30 mM KCl, 10 mM K₂SO₄, 10 mM CaCl₂, 1 mM MgSO₄, and 0.4 M mannitol. Mannitol was included to lower the water potential of the medium to the most negative value measured *in vivo* (1). A previous study of H⁺ fluxes during DD (3) revealed that motor cells do not acidify the medium during their swelling phase unless mannitol is present.

The pH of the bathing solution was monitored continuously for 45 or 72 min, as described in Lee and Satter (3). Changes in pH of the medium provide an indication of H⁺ fluxes (see "Discussion" section). O₂ was bubbled through the solution continuously. All of these manipulations were carried out under dim green safelight. Each graph shows data from a typical experiment selected from three replicates.

RESULTS

Effects of Temperature on pH Changes in the Medium Bathing Excised Motor Tissue during Leaflet Opening. At h 5 of DD, extensor cells start to swell, flexor cells start to shrink, and the pulvinus starts to open (7). When strips of motor tissue were excised and incubated in bathing solutions at this time, extensor tissue acidified the solution at 26 and 30°C with a higher rate at 30°C, and alkalinized the solution at 18 and 22°C with a lower rate at 22°C (Fig. 1a). Flexor tissue alkalinized the solution at all temperatures tested, at a rate that increased slightly with increase in temperature (Fig. 1b).

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² Abbreviation: DD, uninterrupted darkness.

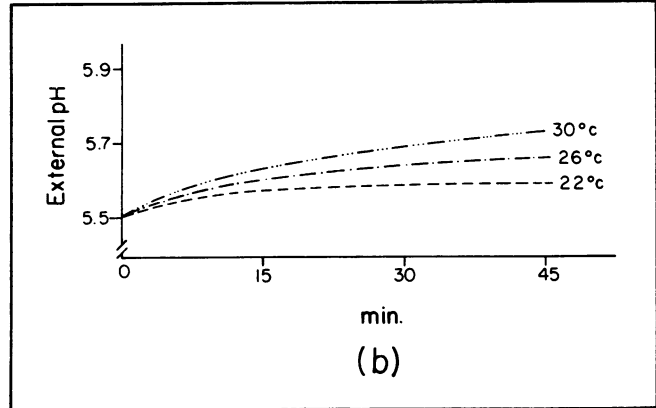
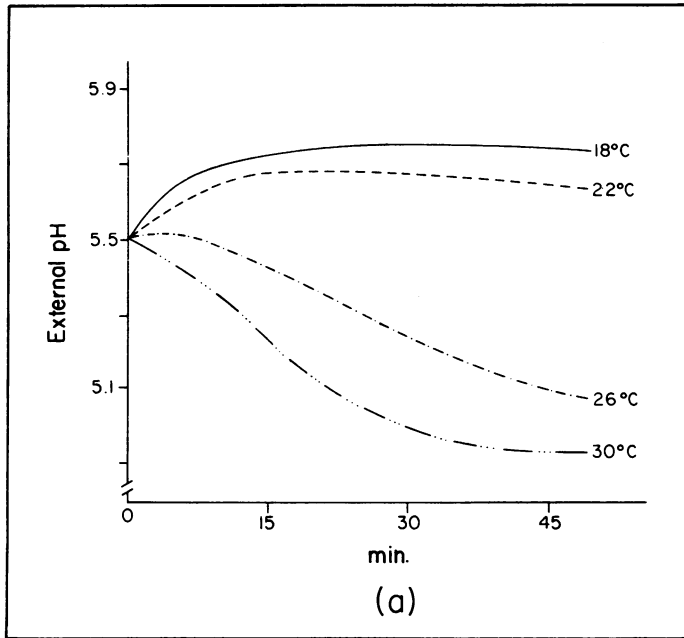


FIG. 1. Effects of temperature on changes in pH of the medium bathing excised (a) extensor and (b) flexor tissue at h 5 of DD. The composition of the bathing solution is given in "Materials and Methods."

Effects of Temperature on pH Changes in the Medium Bathing Excised Motor Tissue during Leaflet Closure. At h 18 of DD, extensor cells start to shrink, flexor cells start to swell, and the pulvinus starts to close (7). When strips of motor tissue were excised and incubated in bathing solutions at this time, extensor tissue alkalinized the medium at a rate that decreased with increase in temperature (Fig. 2a). Flexor tissue initially acidified the solution at a rate that increased with temperature; however, the pH changes reversed direction at all three temperatures: the higher the temperature, the earlier reversal occurred, and the higher the pH at reversal. By 45 min, net acidification was maximal at 18°C and minimal at 30°C.

DISCUSSION

Relationship between pH Changes and H⁺ Fluxes. In earlier studies that utilized methods similar to those described herein, we found that anaerobiosis or H⁺ pump inhibitors prevented acidification of the medium bathing flexor cells, indicating that acidification depends upon H⁺ secretion by the pump (3, 8). However, other processes in addition to H⁺ fluxes may affect

the pH of the medium. Some of these processes were discussed in earlier papers and considered to have minimal effect, although leakage of organic anions from the tissue may raise the pH slightly (2, 3, 8). In addition, exchange of H⁺ with cations in the cell wall might contribute to the pH patterns during the first few minutes of the incubation period.

Net H⁺ flux, the major process responsible for change in pH of the medium after the first few minutes, depends upon a dynamic equilibrium between H⁺ secretion through the pump and H⁺ uptake through several pathways (3, 8), including a H⁺/sucrose symporter (4), a H⁺/Cl⁻ symporter (8), and channels or other pathways for H⁺ diffusion. Of all the processes contributing to the pH change, H⁺ secretion is likely to be most sensitive to temperature, since it requires the synthesis of ATP or some other energy-rich compound. H⁺/sucrose and H⁺/Cl⁻ co-transport may also be enhanced by increase in temperature, since they are active processes. Although release of CO₂ produced during respiration may be elevated by high temperature it is unlikely that it affected the pH of the medium significantly, since the rate at which O₂ was bubbled through the medium was four orders

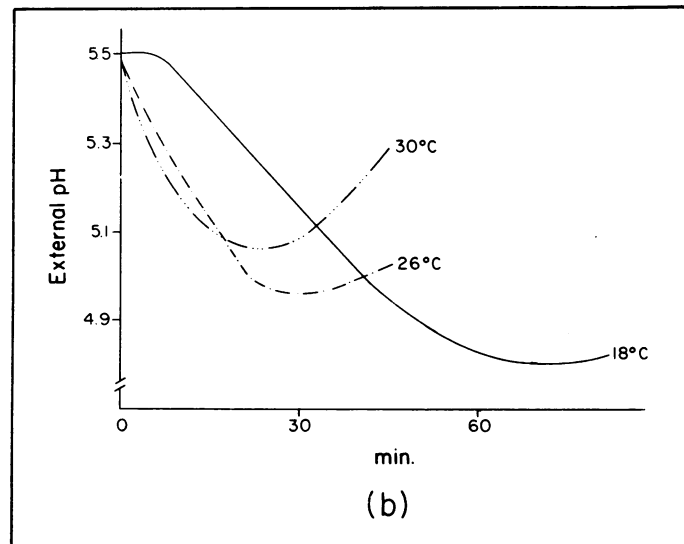
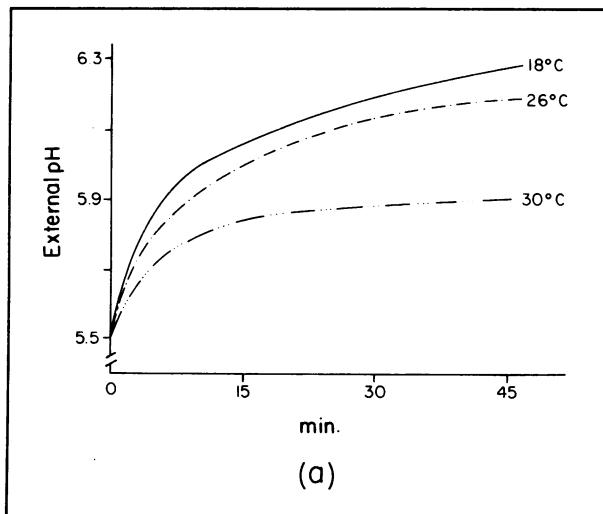


FIG. 2. Effects of temperature on changes in pH of the medium bathing excised (a) extensor and (b) flexor tissue at h 18 of DD. The composition of the bathing solution is given in "Materials and Methods."

of magnitude greater than the rate of CO₂ release at 27.5°C, and thus CO₂ would be replaced very rapidly by O₂ (3).

Comparison of H⁺ Fluxes in Extensor and Flexor Tissue. The responses of extensor and flexor cells to temperature alteration differed from one another when each type of cell was in its swelling phase (compare extensor cells during leaflet opening, Fig. 1a, with flexor cells during leaflet closure, Fig. 2b) and when each was in its shrinking phase (compare extensor cells during closure, Fig. 2a, with flexor cells during opening, Fig. 1b). We interpret patterns of H⁺ flux by extensor cells during opening to indicate that H⁺ secretion increased with temperature and predominated over H⁺ uptake at 26 and 30°C, but at lower temperature, especially at 18°C, the rate of H⁺ secretion was so low that it was masked by H⁺ uptake. H⁺ flux patterns by flexor cells during closure are more difficult to interpret. Initially, high temperature stimulated H⁺ efflux, but after 20 min at 30°C or 70 min at 18°C the pH curve changed direction, indicating that increase in temperature now depressed H⁺ secretion and/or promoted H⁺ uptake (Fig. 2b). Satter *et al.* (8) recently reported a similar response for *Samanea* flexor tissue during dark-induced closure at different temperatures. Their findings may be relevant to this study, since dark-induced closure and rhythmic closure have many characteristics in common. Based on inhibitor studies, they attributed the initial release of H⁺ to a H⁺ pumping ATPase, and the reversal to cessation of pump activity accompanied by H⁺ uptake through various pathways. Why H⁺ pump activity ceased and why it stopped earlier at high temperature was unresolved, although a timing mechanism was suggested.

Both extensor and flexor cells took up H⁺ during their shrinking phases. However, H⁺ uptake by extensor cells decreased with increase in temperature (Fig. 2a), while H⁺ uptake by flexor cells increased with increase in temperature (Fig. 1b). The reason for these differences is not clear. Perhaps H⁺ uptake by extensor cells during closure occurs by a process that is relatively insensitive to temperature (*e.g.* diffusion) but uptake is partially masked by H⁺ secretion at high temperatures. H⁺ uptake by flexor cells during closure, by contrast, may occur by a process that is promoted by high temperature and it may mask any stimulation of H⁺ secretion as the temperature is raised. We previously reported a H⁺ uptake mechanism in flexor cells that appears to utilize metabolic energy (3), and it may be related to the tem-

perature sensitive H⁺ uptake system suggested by data in Figure 1b. This same temperature sensitive H⁺ uptake system may also function during rhythmic closure, and may contribute toward the pH patterns in Figure 2b.

H⁺ Flux and Leaflet Movement. High temperature promotes the opening and reduces the closure of *Samanea* leaflets (7). If the proton-motive force established by the H⁺ pump energizes the accumulation of K⁺ and Cl⁻ in swelling cells and therefore is the basis for rhythmic pulvinar movement (3), high temperature should (a) accelerate H⁺ secretion by extensor cells during opening, and (b) diminish H⁺ secretion by flexor cells during closure. The responses reported in this paper are consistent with these predictions and support the hypothesis. The effects of temperature on H⁺ uptake are small compared to those on H⁺ secretion, but are also consistent with a chemiosmotic hypothesis; high temperature (a) promoted H⁺ uptake by shrinking flexor cells, which may enhance opening; and (b) diminished H⁺ uptake by shrinking extensor cells, which may contribute to slow and incomplete closure. Thus data in this paper provide a new interpretation at the cellular level for the effects of temperature on leaflet movement in *Samanea* (7) and the related legume *Albizzia julibrissin* (5).

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