Rapid Movements of Plants Organs Require Solute-Water Cotransporters or Contractile Proteins

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Plant organs such as leaves or petals move as a result of changes in the shape and/or the volume of motor cells. In a similar manner, stomata open and close when the turgor in the guard cells changes. The time scale of such movements ranges from several milliseconds to hours. The most detailed studies of cellular movement have been done with stomata. Stomatal opening requires the uptake of $K^+$ by guard cell, and the presence of $K^+$ channels in their plasma membranes and tonoplasts that mediate this $K^+$ flux (Fischer, 1968). If water follows $K^+$ flux by osmosis, then the rate of flux would determine the rate of volume change (Schroeder et al., 1984). This explanation is frequently used as a paradigm for other nastic movements (Moran et al., 1990; Fleurat-Lessard et al., 1997; Moshelion and Moran, 2000). For example, in *Mimosa pudica*, the observed potassium exchanges and the nature of the anatomical structures of pulvinule and pulvinus have led to suggestions that movements are the result of modifications in the volume of motor cells at the base of each leaflet and leaf by a “lever” effect. However, none of the movements in plants is really understood at the molecular level. Here, we discuss the constraints brought to the paradigm of movement by considering the values of cell membrane transport parameters and the characteristic time of the movement.

THE RATE-LIMITING STEP OF VOLUME CHANGE IS DETERMINED BY A FLUX OF OSMOTICUM BETWEEN THE CELLULAR COMPARTMENT AND SURROUNDING MEDIUM

If water cannot be actively transported across cellular membranes, the water flux can be expressed from thermodynamic considerations (Nobel, 1999) by:

$$J_w = P_{osm} \left[ \sigma V_w(C_i - C_o) + \frac{V_w P_t}{RT} \right]$$

In this equation, $P_{osm}$ is the osmotic water permeability of the cell. When the osmoticum of the cell is mainly due to one solute, the reflection coefficient $\sigma$ expresses the selectivity of the cell membrane for this solute. It is zero when solute and water move at the same velocity across membranes; it is unity when the solute does not cross the membrane. $P_t$ is the turgor pressure (MPa), in reference to the external medium, $c_i$ and $c_o$ are values of the concentrations in osmoticum (mOsmol) of the external solution and inside the cell. $V_w$ is the partial molal volume of water ($18.05 \times 10^{-6}$ m$^3$ mol$^{-1}$ at 20°C) and at 20°C.

$$\frac{RT}{V_w} = 135 \text{ MPa}$$

A cell will keep a constant volume as long as the water flux is zero, i.e. when there is a balance between the concentration term and the turgor pressure term. If the reception of a signal by a cell resulted in the sudden opening of channels allowing the release of osmoticum, from Equation 1 it would create a water flux:

$$J_w = P_{osm} \frac{\Delta \sigma}{\sigma_{eq}} \left[ \frac{V_w P_t}{RT} \right]_{eq}$$

In this equation, $\Delta \sigma$ is the reduction of the reflection coefficient at equilibrium $\sigma_{eq}$ induced by channel opening. For some cells, NaCl may be considered as a non-permeant species and $\sigma_{eq}$ is close to unity (Steudle and Henzler, 1995). Reflection coefficients smaller than unity were measured previously on entire roots for nutrient salts such as KCl and KNO$_3$, but even in this case, cells were assumed to behave as perfect osmometers, the reduction in $\sigma$ being explained by the lower reflection coefficient of parallel pathways (Steudle, 2000). In the stomata and pulvinar cells, KCl is the main osmoticum and the water flux induced by Equation 2 will remain small as long as the variations in $\Delta \sigma$ can be neglected.

Although we lack information about the changes of $\sigma$ following signal perception, we can nevertheless estimate the upper limit for the rate of volume change based on the known values of potassium fluxes in cells. The efflux of water from a cell with surface area $A$ and volume $V$ is due to a decrease in the concentration $c_i$ of osmoticum through the efflux $J_w$ of species $s$ (e.g. KCl) across $A$. The change of the...
mass $m_i$ of osmoticum ($m_i = c_i V$) allows one to calculate the rate of volume change:

$$-\frac{dV}{dt} = \frac{J_s A}{c_i} + \frac{V d c_i}{c_i} dt$$  \hspace{1cm} (3)$$

We can use Equation 3 to obtain an upper limit for the maximum rate of change for $V$. The second term on the right side of Equation 3 will be negative as long as the rigidity of the wall remains constant or at least is not increased during the reduction in cell volume (we discuss this point later). We then consider the value zero as an upper limit for this term. A maximum for the first term on the right side of Equation 3 is obtained by using a minimum value $c_i \text{min}$ for $c_i$ and the maximum value $A_0$ for $A$. When the cell is in its resting state, prior to receiving a stimulus, $c_i$, $V$, and $A$ have their initial values $c_{i0}$, $V_0$, and $A_0$. If a stimulus creates a constant $J_{s0}$ at time $t_0$, the maximum rate of change for $V$ is:

$$\left[-\frac{dV}{dt}\right]_{\text{max}} = \frac{J_{s0} A_0}{c_i \text{min}}$$  \hspace{1cm} (4)$$

Equation 4 allows calculation of the minimum time required to modify the volume of a cell. In the case of stomata, following Schroeder et al. (1984) and Assmann et al. (1985), we consider that for a spherical guard cell with initial radius $R_0 = 7 \times 10^{-6}$ m, the flux of potassium ions responsible for stomatal movement is $J_{s0} = 2 \times 10^{-7}$ mol m$^{-2}$ s$^{-1}$. The osmolarity of the cell is not known, but even if we consider a cell with an osmolarity as low as 100 mOsmol, a significant change in $V$, for example 10%, could not occur in less than 100 s. In the case of M. pudica, the maximum currents measured on protoplasts from motor cells correspond to current densities that may be 10 times larger than those found in guard cells (Moran et al., 1990; Stoeckel and Takeda, 1995), but the volume of these cells decreases by up to 25% during leaflet closure (Fleurat-Lessard et al., 1997). Several tens of seconds would still be required for such a volume change. Values of 100 s are compatible with a stomatal closure requiring several minutes, but to explain the rapid movement of a leaf within 1 s (Fleurat-Lessard et al., 1997) by a flux of osmoticum, we would have to assume very large values for $J_{s0}$, i.e. a large decrease in $\sigma$ and/or very low values for $c_i \text{min}$. Measuring a transient decrease in $\sigma$ is probably beyond technical capabilities, but patch-clamp measurements could allow detection of a sudden change in the cell conductance resulting from the simultaneous opening of many anion and cation channels. However, according to the present data, a 10% volume change in a few seconds is difficult to explain by a fast uptake or release of K$^+$ ions between the cell and its surroundings. We suggest another mechanism based on the hypothesis that molecular coupling between water and a solute species occurs in cellular membranes.

**THE RATE-LIMITING STEP OF VOLUME CHANGE IS DETERMINED BY AN EFFLUX OF WATER FROM THE CELLULAR COMPARTMENT**

We propose that apart from the passive flux described by Equation 1, the plasma membrane of the pulvinar cells may contain cotransporters or “water pumps” able to create an uphill transport of water molecules into the cell (Fig. 1) with a rate $J^+_{sw}$ (volume of water per unit of time and membrane surface; m$^3$s$^{-1}$). If we start from an equilibrium situation, when pumps are “switched on,” they cause water molecules to enter at a constant rate into the cell, which increases turgor. This change in turgor corresponds to a driving force that expels water molecules through the passive leaks ($P_{os}$). When the magnitude of the passive efflux reaches that of the water pump, there is no longer a net increase in the cell water content and turgor reaches its maximum value. As long as the “state parameters” ($P_{os}$, $\sigma$, and the rate of water pump) remain constants, the driving force that has been created by the pump also keeps a constant value. If, for some reason, $P_{os}$ is suddenly increased 10 times, Equation 1 indicates that the driving force will result in a 10-fold increase in the passive water efflux. This water efflux is now 10 times larger than the pumped water influx so there is a rapid drop in cell volume and turgor and this large water efflux is only transient. We can quantify the reasoning to explain the volume change in pulvinar cells.

Prior to signal perception, the cell is in a steady state. Its membrane has a low water permeability ($P_{os}$) and a passive water flux ($J_{sw}$) given by Equation 1, which is equal to the flux of pumped water ($J^+_{sw}$). The driving force that is created in this steady state is simply the ratio $J^+_{sw}$ to $P_{os}$. If, following stimulation, the water permeability of the plasma membrane transiently increases by rapid opening of aquaporins, resulting in a higher value of $P_{os}$ then...
the new water flux out of the cell $J^m_v$ can be expressed as the product of this new permeability by the force:

$$J^M_v = \frac{P^M}{{P^m}} \times J^v$$   \hspace{1cm} (5)

We will consider that the net efflux of water in the stimulated state, $J^m_v - J^v$, is very close to $J^m_v$, because the permeability in this state can be assumed to be much larger than that in the resting state. The initial rate of volume change is determined by the water permeability of the cell (Ramahaleo et al., 1999). For a spherical cell, the following holds:

$$\frac{d(V/V_0)}{dt} = -\frac{3}{v} J^M_v$$   \hspace{1cm} (6)

For a pulvinar cell with 20-μm initial diameter, a 20% change in volume in 1 s would correspond to $J^m_v = 7 \times 10^{-7}$ ms$^{-1}$, about $3.7 \times 10^{-5}$ mol m$^{-2}$ s$^{-1}$.

The active water flux ($P^m$) required to create this flux will be determined by the ratio between maximum and minimum permeability. The lowest $P^m$ that we measured on isolated protoplasts was around 1 μm$^{-1}$ and the largest was 1,000 μm$^{-1}$ (Ramahaleo et al., 1999), giving a maximum value of 1,000 for this ratio. This would correspond to a $J^v$, value of about $3.7 \times 10^{-5}$ mol m$^{-2}$ s$^{-1}$. Previous authors (Zeuthen and Stein, 1994) have invoked a “water pump” to account for certain physiological processes in animals. They describe specific cotransporters that couple several hundred water molecules to the transport of a single Glu or sodium molecule (Zeuthen et al., 1997; Meinhild et al., 1998), creating active water fluxes of about $10^{-3}$ mol m$^{-2}$ s$^{-1}$ in oocytes (MacAuley et al., 2001). So, provided that a substantial coupling also exists in plants, the model allows volume changes in the 1-s range.

In the case of M. pudica, the smaller $P^m$ value that we measured on protoplasts from pulvinar cells was about 1 μm$^{-1}$ (data not shown). This very low value could be increased 1,000-fold after stimulation by the opening of aquaporins. It is unfortunate that the technique we use does not allow $P^m$ measurements in a transient state.

To get an idea of the feasibility of the water pump, we can estimate the energetic cost for a cell during the steady state with a low $P^m$ of 1 μm$^{-1}$. In the case of the pulvinar cell described above, it would correspond to a water flux of $7 \times 10^{-10}$ ms$^{-1}$ and a force of about $10^5$ Pa. The second principle of thermodynamics implies a minimum value for the metabolic energy that has to be supplied to the pump, which is the product of the flux by the force: $7 \times 10^{-10}$ ms$^{-1} \times 10^5 Pa = 7 \times 10^{-4}$ J m$^{-2}$ s$^{-1}$. We do not know the rate at which the energy is available in a pulvinar cell, but there are some data concerning giant algal cells (Lütgge and Pitman, 1976). For Chara australis, Hope and Walker (1975) calculated from the flux of CO$_2$ that photosynthesis produced about 0.2 Jm$^{-2}$ s$^{-1}$, and from the flux of O$_2$ that respiration produced $3 \times 10^{-2}$ Jm$^{-2}$ s$^{-1}$, assuming a value of $3.3 \times 10^1$ J mol$^{-1}$ for the free energy of ATP hydrolysis. If comparable values were valid for the cells in which active water fluxes are found, the consumption of energy by the water pump would then be a small proportion of that available to the cell.

In our model, the rate of cellular movement is determined by the water efflux induced by a very rapid change in $P^m$ monitored by a fast and transient opening of aquaporins. After the cellular movement, the aquaporins close and the force creating high initial pressure will be restored by the continuous action of the uphill water cotransporters. There is another way to quickly modify water flux according to Equation 1: A mechanical stimulus could increase $P^m$ very rapidly by modification of the external stress applied to the cell wall. However, the water flux would still be limited by the permeability of the cell membrane to water. Movement in 10 ms, implying a water flux 100 times higher than that previously used in Equation 6, would require permeability orders of magnitudes too high, as already indicated (Hill and Findlay, 1981). Then it seems unlikely to explain movements with a time scale of a few tens of millisecondes (Dionaea muscipula and Utricularia vulgaris) by an exchange of water between a cell and its surroundings.

The questions raised by the fast signal transduction in plants from the external signal to the cellular response have already been examined (Sibaoka, 1969). A mechanism similar to that of muscle contraction could explain these movements because it does not rely on the relatively “slow” changes in osmoticum between the cell and its surroundings: Only contractile proteins that modify the shape of the cell (e.g., a change in the ratio length/diameter) without the need to change its volume are involved. (Kameyama et al., 2000) reported that in M. pudica, bending of the petiole was correlated with a change in the level of phosphorylation of actin, suggesting that contractile proteins may be involved in plant movements. In summary, by defining the limits of the rate of volume change at the cellular level we conclude that movement in plants in the 1-s range are not consistent with the prevailing explanation that water follows osmoticum by osmosis. Such rapid movements require either an uphill transport by solute-water cotransporters across a membrane with a regulated osmotic permeability, or a mechanism of cell shape change at constant volume.

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LITERATURE CITED


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