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Simulation of the Light-Induced Oscillations of the Membrane Potential in *Potamogeton* Leaf Cells

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Summary. An attempt has been made to simulate the light-induced oscillations of the membrane potential of *Potamogeton lucens* leaf cells in relation to the apoplastic pH changes. Previously it was demonstrated that the membrane potential of these cells can be described in terms of proton movements only. It is hypothesized that the membrane potential is determined by an electrogenic H^+-ATPase with a variable H^+/ATP stoichiometry. The stoichiometry shifts from a value of two in the dark to a value of one in the light. Moreover, this H^+ pump shows the characteristics of either a pump or a passive H^+ conductance: the mode of operation of the H^+ translocator is considered to be regulated by the external pH. The pump conductance is assumed to be dominant at low or neutral pH, while the passive H^+ conductance becomes more significant at alkaline pH. The pH dependence of the transport characteristic is expressed by protonation reactions in the plasma membrane. The proposed model can account for most features of the light-induced oscillations but not for the absolute level of the membrane potential.

Key Words membrane potential · simulation model, H^+-ATPase · H^+ conductance · *Potamogeton lucens*

Introduction

Although the effect of light on the membrane potential (E_m) of photosynthetically active plant cells has been studied by a number of authors (Vredenberg, 1974; Felle & Bentrup, 1976; Prins, Harper & Higinbotham, 1980; Mimura & Tazawa, 1986; Spanswick, 1990), reports concerning a more quantitative description of the response are scarce. Stolarek, Karcz and Pietruszka (1988) mathematically analyzed the effect of light on E_m in *Cucurbita pepo* L. The result led to the conclusion that the bioelectric response was composed of a hyperpolarizing (the plasmalemma H^+-ATPase) and a depolarizing (a passive Cl^- conductance) component.

Hansen and co-workers (1987) approached the problem from a different viewpoint. By measuring different physiological parameters in the *Nitella* cell (e.g., E_m, the chlorophyl fluorescence and the oxygen evolution) and by comparing the time constants of their light-induced changes, they were able to relate these time constants to different biochemical events.

The present study deals with a simulation of the light-induced oscillations of E_m in cells of *Potamogeton* in relation to the observed changes in leaf surface pH. The light-induced pH polarity in leaves of *Potamogeton lucens* and *Elodea densa* has previously been documented in detail (Prins et al., 1982; Elzenga & Prins, 1989; Miedema & Prins, 1992). One of the mechanisms causing the observed polarity is an increased proton permeability (P_{H^+}) of the upper plasma membrane (Miedema & Prins, 1991; Miedema, Felle & Prins, 1992). The increase of P_{H^+} may facilitate the H^+ re-entry at this side of the leaf.

The mechanism of passive H^+ transfer across biological membranes is poorly understood. The phospholipid bilayer itself shows an intrinsic, almost pH-independent P_{H^+} (Deamer & Nichols, 1989). Another characteristic is that, compared to other small cations, P_{H^+} of the lipid bilayer is anomalously high. An explanation for the high P_{H^+} is given by a theory based on H^+ conduction along so-called Hydrogen Bonded Chains (HBC). The HBC may be formed by water molecules spanning the membrane. The same mechanism of H^+ conduction may play a role in H^+ conduction through transport proteins. In this case the HBC may be formed by a network of, for instance, amino acid residues and water molecules (Deamer & Nichols, 1983, 1989; Nagel & Tristam-Nagle, 1983). Despite the suggested presence of a dominating H^+ conductance in several types of biological membranes (*Elodea* and *Potamogeton:* Miedema & Prins, 1991; Miedema et al. 1992; *Chara:*

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Bisson & Walker, 1980; Walker, 1983; snail neurons: Thomas & Meech, 1982), until now only one H^+ channel has been identified and characterized: the CF_o subunit of the chloroplast ATP-synthase (Althoff, Lille & Junge, 1989; Wagner, Apley & Hanke, 1989).

In this paper, we try to explain the observed light-induced oscillations of the *Potamogeton* E_m, in a model based on the assumption that the active H^+ efflux as well as the passive H^+ flux are accomplished by the same membrane transporter, namely the plasmalemma-bound H^+-ATPase. Lucas and Fisahn (1989) suggested a similar behavior of the *Chara* plasmalemma H^+-ATPase. A second assumption is that the H^+-ATPase shows a variable rather than a fixed stoichiometry: the H^+/ATPstoichiometry is related to the calculated proton motive force (pmf) across the membrane.

The model has to explain the observed pH dependence of P_{H^+}. Therefore, the mode of operation of this putative transporter is assumed to be regulated by the extracellular pH. At low external pH, the transporter functions as an electrogenic pump, while with an increasing pH, the passive conductance becomes more and more significant. Consequently, at strong alkaline pH the complex should behave as a passive H^+ channel. To find a convenient mathematical expression for the regulation of the H^+translocator by the external pH, we have assumed that such a regulation occurs via protonation reactions in the plasmalemma.

Materials and Methods

Culture Conditions

P. lucens was grown indoors in tanks on a clay substrate covered with a thin layer of sand to prevent solubilization of the clay particles. The tanks were filled with demineralized water and the subsequent nutrient concentrations determined: $K^+ < 0.1$ mM; $Na^+ < 0.1$ mM; $Ca^{2+} = 0.5$–1.3 mM; $Cl^- < 0.1$ mM. The pH varied between 7.5 and 8.5; the temperature between 19 and 21°C. At the start of the experiment, the leaf was carefully rinsed with distilled water to clean the surface from epiphytes and marl. The light regime was 12 hr light, alternated with 12 hr dark. The high pressure mercury lamps (Philips, HPLN 400W) radiated only minimal UV rays. The irradiance at the water surface was 90 μmol/m^2.

Experimental Setup

The experimental setup for the simultaneous measurement of leaf surface pH and membrane potential has been described in a previous study (Miedema & Prins, 1991). A computer linked to this setup sampled the signals from the recording electrodes at 15-sec intervals. The pH data were used for the simulation of E_m.

The experimental solution contained (mM) 4.5 $CaCl_2$, 2.5 KCl and 0.5 $KHCO_3$ adjusted to pH 6.5. The experiments were performed at an ambient room temperature of 20°C. The irradiance during the experiments was 100 μmol/m^2.

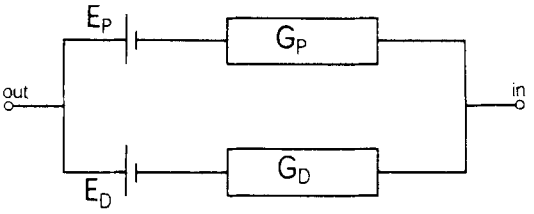

Fig. 1. The equivalent circuit for a passive conductance (G_D) and an electrogenic pump (G_P) working in parallel. E_D represents the Nernst or diffusion potential; E_P the electromotive force (emf) of the pump.

The Model

The aim of the model was to describe the light-induced oscillations of E_m as a function of the surface pH on both sides of the *Potamogeton* leaf and to understand to what extent these oscillations can be ascribed to external pH effects on the H^+ and pump conductance of the plasma membrane. For this purpose, the pH at the leaf surface was considered to be an independent parameter: the model does not account for the observed pH changes; rather, it predicts the changes in E_m caused by changes of the apoplastic pH. We assumed that E_m was at steady-state at any pH. The adjustment of E_m to the external pH was thus considered to occur almost instantaneously compared to the rate of pH change itself.

In intact leaves of *Potamogeton* and *Elodea*, E_m is almost insensitive to the external K^+ concentration up to a value of 50 mM (Miedema et al., 1992). An electrogenic H^+-ATPase on the lower and a passive H^+ conductance on the upper side of the leaf dominate E_m. The proton pump can be considered as a voltage source in series with a conductance (Keifer & Spanswick, 1978; Spanswick, 1981; Takeshinge, Shimmen & Tazawa, 1985). The equivalent circuit for such an electrogenic H^+pump and a passive H^+ conductance working in parallel is given in Fig. 1. The steady-state membrane potential (E) is then given by:

$$E = \frac{E_p G_p + E_{H^+} G_{H^+}}{G_p + G_{H^+}} \quad (1)$$

wherein E_p represents the electromotive force (emf) of the pump, G_p the pump conductance, E_{H^+} the diffusion potential of H^+, and G_{H^+} the passive H^+ conductance.

In the model, the pump and channel conductances are ascribed to the same membrane transporter, whose mode of operation is regulated by the external pH. Protonation reactions are introduced to express this pH dependence. The chemical nature of the compound or chemical group which is susceptible to protonation is not essential to the model. Possible candidates are, for instance, carboxyl or amino groups of amino acids.

For a single protonation the Henderson-Hasselbalch equation gives the ratio of deprotonated and protonated compound (R/RH^+) in relation to pH and pK:

$$\frac{R}{RH^+} = 10^{(pH-pK)} \tag{2}$$

In the model it is proposed that n protonation steps are involved in the regulation of each transporter. Then, it can be derived that the ratio between the completely deprotonated (MT) and protonated membrane transporter (MTH_n) is given by:

$$\frac{MT}{MTH_n} = 10^{(pH-pK_1)}10^{(pH-pK_2)} \cdot \cdot \cdot 10^{(pH-pK_n)} \tag{3}$$

For simplicity, it is assumed that all involved active sites (n) of a single transporter have the same pK value. This reduces Eq. (3) to:

$$\frac{MT}{MTH_n} = 10^{n(pH-pK)} \tag{4}$$

The pH dependence of MT/MTH_n is applied to a population of transporters. Consequently, MT/MTH_nrepresents the ratio of the number of transporters occupying the channel or pump state. The ratio of channel and pump conductance of the plasma membrane (G_{H^+}/G_p) is postulated to be directly proportional to MT/MTH_n. As at higher pH MT/MTH_n increases and the channel characteristic will become more significant, G_{H^+}/G_p is proportional to MT/MTH_n and not to MTH_n/MT.

On the microscopic scale, the ratio of the channel and pump conductance of a *single* transporter (g_{H^+}/g_p) will be expressed by β. A single complex should behave either as a pump or as a channel, reflecting the two possible protonation states in which the transporter can exist. The value of β is assumed to be pH independent. G_{H^+}/G_p will also be proportional to β, and will be given by:

$$\frac{G_{H^+}}{G_p} = \beta\frac{MT}{MTH_n} \tag{5}$$

Substitution of Eq. (4) into Eq. (5) results in an expression which reflects the pH dependence of G_{H^+}/G_p:

$$\frac{G_{H^+}}{G_p} = \beta 10^{n(pH-pK)} \tag{6}$$

It should be realized that, whether the pH dependence is applied to the population of transporters or, alternatively, to each individual enzyme, the final result should be identical.

An anatomical difference between both sides of the leaf is introduced by the assumption that a fraction α of transporters is located on the upper side of the leaf while a fraction 1-α is located on the lower side. The equivalent circuit of Fig. 1 is then replaced by the one depicted in Fig. 2. This circuit is based on the assumption of a negligible plasmodesmatal resistance between the two cell types; only then can the H^+ transporters in either plasma membrane be thought to operate in parallel. Despite efforts to detect a possible difference in E_m between the two cells, such a difference has never been found. For the same reason, information concerning which cell type actually has been impaled by the electrode is not relevant to the description of the E_m response. According to Fig. 2, E is expressed by:

$$E = \frac{\alpha(E_pG_p + E_{H^+}G_{H^+})_{up} + (1-\alpha)(E_pG_P + E_{H^+}G_{H^+})_{low}}{\alpha(G_P + G_{H^+})_{up} + (1-\alpha)(G_p + G_{H^+})_{low}} \tag{7}$$

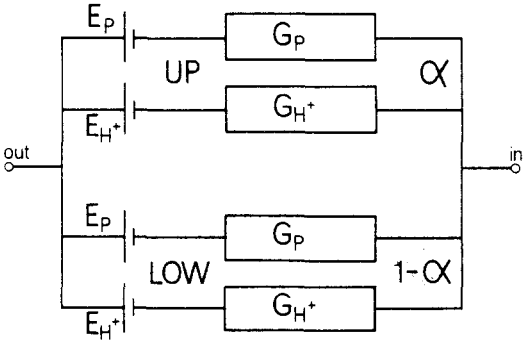

Fig. 2. The proposed equivalent circuit for an intact *Potamogeton* leaf. The circuit is based on the assumption that a fraction of α of the H^+ transporters, operating either as a pump or as a channel, is located on the upper leaf surface and a fraction of $1-\alpha$ on the lower surface.

Eq. (7) contains the values of G_p and G_{H^+}. However, the model requires the ratio of both conductances rather than the absolute values. Unfortunately, division by, for instance, $G_{p,up}$ introduces the unknown ratio $G_{H^+,low}/G_{p,up}$. One possible solution may be to replace this expression by the product of $G_{H^+,low}/G_{p,low}$ and $G_{p,low}/G_{p,up}$, possibly the latter term can be related to the ratio of pump stoichiometry at either leaf side. We chose an alternative approach: the terms $(G_p + G_{H^+})_{low}$ and $(G_p + G_{H^+})_{up}$ in Eq. (7) were assumed to be related according to:

$$\frac{(G_P + G_{H^+})_{up}}{(G_P + G_{H^+})_{low}} \approx \beta\frac{\alpha}{1-\alpha} \tag{8}$$

It is realized that this expression is just an approximation. In fact, this relation only holds under steady polar conditions as, according to the model, only then can all the transporters be assumed to be in the H^+ or in the pump state on the upper and lower side of the leaf, respectively. Substitution of Eq. (8) and rearranging Eq. (7) renders:

$$E = \frac{\left(E_p + E_{H^+}\frac{G_{H^+}}{G_P}\right)_{up}}{\left(1 + \frac{(1-\alpha)^2}{\beta\alpha^2}\right)\left(1 + \frac{G_{H^+}}{G_p}\right)_{up}} + \frac{\left(E_p + E_{H^+}\frac{G_{H^+}}{G_p}\right)_{low}}{\left(1 + \frac{\beta\alpha^2}{(1-\alpha)^2}\right)\left(1 + \frac{G_{H^+}}{G_p}\right)_{low}} \tag{9}$$

Finally, expressions for E_{H^+} and $E_p$are required. E_{H^+} is given by:

$$E_{H^+} = 2.303\frac{RT}{F}(pH_{cyt} - pH_{out}) \tag{10}$$

To obtain an expression for E_p it has to be realized that the H^+-ATPase is thermodynamically constrained by the change in free energy of ATP hydrolysis (ΔG_{ATP}) in relation to the value of $\Delta\mu_{H^+}$ across the membrane. Then the pump potential E_p becomes:

$$E_p = \frac{\Delta G_{ATP}}{Fs} + E_{H^+} \quad (11)$$

wherein F represents Faraday's constant and s the stoichiometry of the H^+ pump. A premise of the model is that the H^+-ATPase at the acidifying lower side of the leaf works near thermodynamical equilibrium, i.e., E_m can be substituted for E_p in Eq. (11). Therefore, the value of s was derived from the experimentally observed proton motive force (pmf) across the membrane: s is given by the ratio between $\Delta G_{ATP}/F$ and pmf, the latter equals $E_m - E_{H^+}$. However, the H^+/ATP stoichiometry was not supposed to exceed the value of two.

To calculate E, Eqs. (6), (10) and (11) have to be substituted into Eq. (9), together with the experimentally obtained leaf surface pH on either side of the leaf.

Results

Experimental Data

Figure 3 shows the light-induced changes of leaf surface pH (*A*), membrane potential (*B*) and the calculated pmf, derived from (*A*) and (*B*), assuming a constant cytoplasmic pH of 7.3. At the end of the light period the pmf across the upper plasmalemma became close to zero, indicating a strongly increased P_{H^+} of the upper plasma membrane.

As a first step in understanding the oscillatory behavior of E_m, the response of E_m was separated into five consecutive phases, and the measured value of E_m was compared with the theoretical values of E_{H^+} and E_p.

To calculate E_{H^+} from Eq. (10) the value of the cytosolic pH (pH_{cyt}) has to be known. Preliminary experiments using pH-sensitive microelectrodes indicated pH_{cyt} to be around 7.3. As already observed in other aquatics (Felle & Bertl, 1986), the light-induced changes in pH_{cyt} appeared to be small. Therefore, in all calculations we used the value of 7.3 and neglected possible light-induced changes.

The calculation of E_p requires an estimation of the H^+/ATP stoichiometry of the pump and $\Delta G_{ATP}/F$. As in the light, under dark conditions, E_m of *Elodea* hardly depolarized at high external K^+ concentrations. In *Elodea* and *Potamogeton* the light responses of both leaf surface pH and membrane potential are very similar. Therefore, it is believed that in the dark E_m of *Potamogeton* is also dominated by E_p rather than by any diffusion potential (*see also* Cruz-Mireles & Ortega-Blake, 1991). Under physiological conditions the value of $\Delta G_{ATP}/F$ may be as high as −570 mV and is believed to be rather constant (Bennett & Spanswick, 1984; Briskin & Reynolds-Niesman, 1991; Raven, 1991). In our calculations the value of E_p was adjusted to the dark-level of E_m. Assuming a stoichiometry of two, we calculated $\Delta G_{ATP}/F$ to be −400 mV.

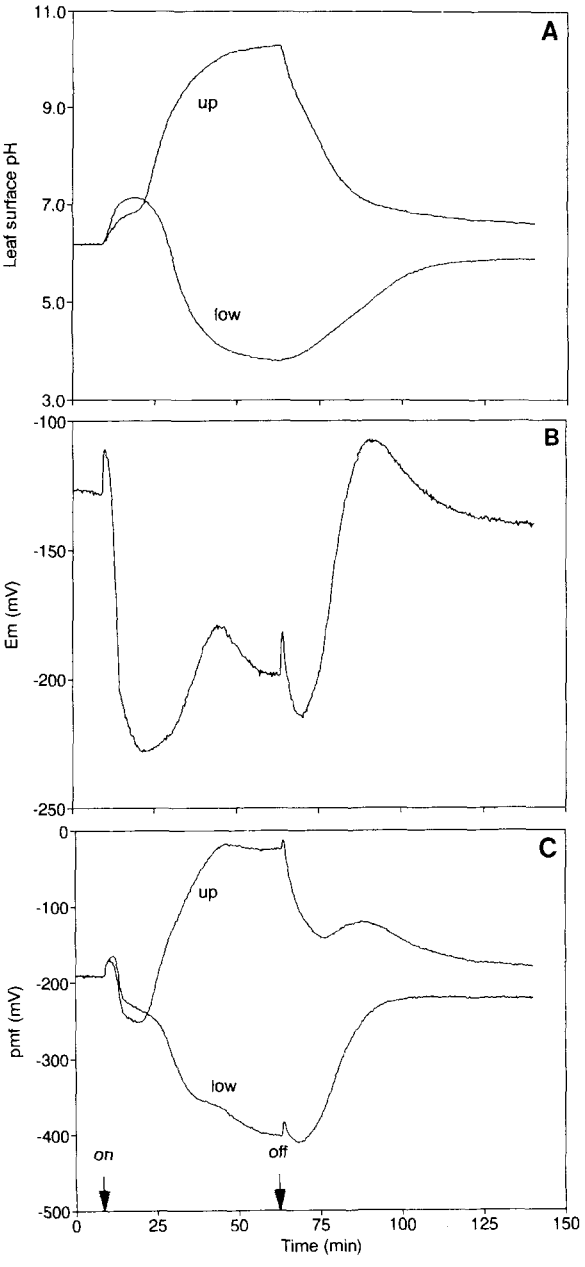

Fig. 3. The recorded light-induced changes of leaf surface pH (*A*) and membrane potential (*B*). The proton motive force (pmf), defined as $\mu_{H^+,in} - \mu_{H^+,out}$, was calculated using the data from *A* and *B* while assuming a (constant) cytoplasmic pH of 7.3 (*C*). *Up* and *low* refer to the morphological upper and lower leaf side; and arrows to light-on and light-off.

The calculated values of E_p and E_{H^+} on either side of the leaf and the recorded E_m are shown in Fig. 4. Owing to the interrelation between pmf and s at the lower leaf side, E_m and $(E_p)_{low}$ almost coincide. The initial very rapid transient depolarization of E_m in phase 1 was not found in the calculated $(E_p)_{low}$. The subsequent hyperpolarization corre-

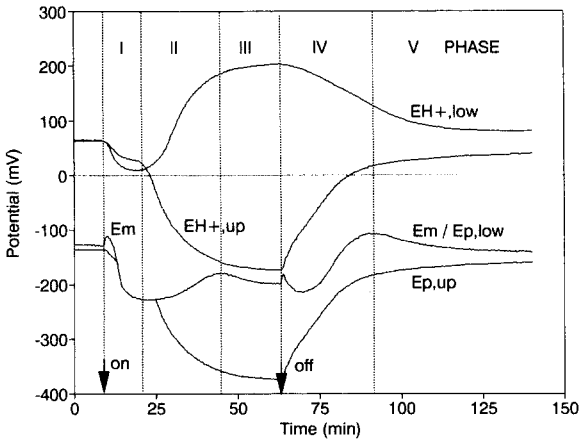

Fig. 4. The light-induced response of the recorded membrane potential E_m and the calculated E_p and E_{H^+} at both sides of the leaf. The values of $\Delta G_{ATP}/F$ and pH_{cyt} were −400 mV and 7.3, respectively. Owing to the proposed interrelation, E_m and $(E_p)_{low}$ almost coincide during the entire interval. $(E_p)_{up}$ coincides with E_m only during the first hyperpolarization after light on. Arrows refer to light-on and light-off.

lated with the hyperpolarization of E_p due to the alkalinizing CO_2 uptake that occurs on both sides during the initial nonpolar phase (Prins et al., 1980). In the second phase there was a clear correlation between the depolarization of E_m and the H^+ influx on the upper side. The depolarizing effect of this H^+ influx was possibly enhanced by the simultaneous depolarizing tendency of $(E_p)_{low}$. During the third phase, the curves of E_m and $(E_{H^+})_{up}$ did not exactly coincide. Nevertheless, the results suggest that in this phase E_m was dominated by $(E_{H^+})_{up}$. The gradual hyperpolarization of E_m during this period may be explained by an efflux of H^+, due to $(E_{H^+})_{up}$ being more negative than E_m.

The fourth phase starts after light-off. During this phase the behavior of E_m was rather complex and more difficult to interpret. The small initial depolarization may reflect the depolarization of $(E_{H^+})_{up}$. The subsequent hyperpolarization indicates a more dominant role of $(E_p)_{up}$. During the fifth and last phase, the dark level of E_m was dominated by E_p; $(E_p)_{low}$ and $(E_p)_{up}$ now tended to attain the same steady-state value.

Simulation of E_m

We used this model to simulate the light and dark induced changes of E_m as determined by the surface pH's near both leaf sides. Recently, Lützelschwab (*personal communication*) reported an uneven distribution of plasmalemma bound H^+-ATPase in *Elodea* leaf cells, using the immunocytolocalization technique (Villalba, Lützelschwab & Serrano, 1991). This enzyme would be more abundant (5–10 times) in the PM of the lower cell layer. Considering this finding, the value of α (fraction of transporters at the upper leaf side) was taken as 0.15. All other parameters, except the stoichiometry of the pump, attained the same value on both sides of the leaf.

The value of β (g_{H^+}/g_p) was obtained from a report by Bisson (1986*a*). She studied the effect of high pH on the membrane conductance (G_m) in cells of *Chara corallina*. Compared with pH 7.5, she measured a five to tenfold increase in G_m at pH 11. To be consistent, according to our model this change in G_m should reflect the change in conductance of a single transporter as almost all transporters are in the H^+ state at pH 11 or in the pump state at pH 7.5. Therefore, β was assumed to be 10.

As already mentioned above, $\Delta G_{ATP}/F$ was set on −400 mV, while a constant cytoplasmic pH of 7.3 was used. Further on, we assumed that two protonation steps (n= 2) are involved in the regulation mechanism of each transporter and that the pK of the involved active sites was 9.5. It is worth noting that the theory of H^+conduction along an HBC predicts a rather strong pH dependence. Maximal rates are expected when both pK and pH are below 5 or exceed 9 (Wagner et al., 1989, and references herein). In this respect, the choice of the pK value of 9.5 in the present model receives some theoretical support.

During H^+ dissociation of identical sites of a protein, the pK of the site will increase after each preceding dissociation. For reasons of simplicity, however, the pK's of the sites were assumed to be identical.

E was calculated according to Eq. (9) and the result is shown in Fig. 5. Despite the differences between the absolute values of the recorded (E_m) and the calculated (E) membrane potential, the oscillating behavior of E_m could be simulated.

Discussion

The Dual Behavior of the Putative Proton Transporter

The present model approaches the problem from a rather phenomenological point of view. As Nagle and Tristam-Nagle (1983) correctly argue, phenomenological theories may appeal to processes for which a physical mechanism is (still) lacking.

Supporting evidence for the existence of a single transporter that exhibits such a dual pump/channel behavior comes from the effects of DES (diethylstil-

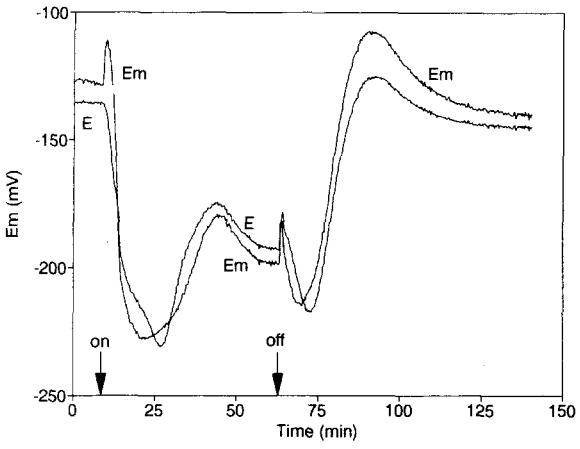

Fig. 5. A comparison between the recorded oscillations of the membrane potential (E_m) and the calculated potential (E) according to the present model during a dark-light transition and vice versa. The chosen parameter values were: $\Delta G_{ATP}/F = -400$ mV; $\alpha = 0.15$; $\beta = 10$; pK = 9.5; $n = 2$ and $pH_{cyt} = 7.3$. Arrows refer to light-on and light-off.

bestrol) and EDAC (1-ethyl-3-(3-dimethylaminodopyl)carbodiimide) on membrane potential and conductance. While the cytoplasmic streaming was unaffected, these compounds inhibited E_m at pH 7.5 and G_m at pH 11 with about the same time course (Bisson, 1986*b*). This similarity in inhibition time course when the cell occupied either the active (or pump) or H^+ state can be interpreted as evidence for the existence of such a transporter.

The observed inversion of the current profile at the *Chara* cell surface may also be interpreted in terms of such a putative transporter (Lucas & Fisahn, 1989; Fisahn & Lucas, 1990). Moreover, in *Chara* the pH banding was voltage insensitive in the range −230 to −130 mV (Fisahn & Lucas, 1991). This finding is in accordance with the assumption used in the present model, that not E_m but the external pH is the main regulating factor in the mode of operation of the transporter.

The pump conductance in the equivalent circuit of Fig. 1 provides a pathway for H^+ out of the cell or vice versa. Despite the lack of experimental evidence, there is no argument based on thermodynamics which forbids such a H^+ movement through the ATPase from the outside to the inside (Kishimoto, Kami-ike & Takeuchi, 1980). The difference between a pump and a carrier or channel is perhaps less distinct than one may expect at first sight. Läuger (1980) already described a pump in terms of a channel with multiple conformational states. Light or, for instance, a phosphorylation, temporarily modify the energy profile of the channel, resulting in the possibility of a pumping mechanism.

Proposing the pump as the pathway for the downhill movement of H^+ instead of the alternative pump/leak model, evades the question whether the pumps are still operating in the alkaline zones of the *Chara* cell surface or on the upper side of the *Potamogeton* leaf. Considering a substantial leak of the membrane for H^+, such a pump action seems rather wasteful (Beilby & Bisson, 1992).

Finally, it has to be stressed that a model consisting of two physically separated transport systems for H^+ efflux and influx would result in identical predictions, provided that external pH affects both conductances in a way similar to the present model. However, a direct relation between pump and channel conductance is then difficult to envisage and, consequently, a mathematical expression, reflecting the supposed coupling, is harder to give.

The Conductance and Stoichiometry of the Pump

Discussions regarding the stoichiometry of the pump are directly related to the question of whether the pump shows the characteristics of a conductance or, alternatively, has to be considered as a constant current source. The value of E_p reflects the potency or the maximal potential which can be generated by the pump, given the physiological and external conditions. At low E_{H^+}, the deviation of E_m from E_p reflects whether the pump is kinetically or thermodynamically controlled. An E_m of −200 mV, while assuming a H^+/ATP stoichiometry of one, implicitly indicates a kinetically controlled pumping rate.

Studies of the H^+/ATP stoichiometry of the plasmalemma H^+-ATPase remain controversial. Even for one species (*Chara*) the question is unclear, stoichiometries of one (Beilby, 1984; Fisahn & Lucas, 1992), as well as of two (Thurstan Smith & Walker, 1981; Kami-ike et al., 1986; Beilby & Bisson, 1992) are reported. Generally, the value of s is derived from observed reversal potentials. A reversal potential of the pump I-V curve of −450 mV, as reported by Fisahn and Lucas (1992), indicates, unambigiously, a stoichiometry of one. However, under most conditions the *Chara* E_m is less hyperpolarized, to about −200 mV. Considering the maximal value of G_{ATP}/F (−570 mV) a stoichiometry of two cannot be excluded. Present understanding of the coupling between ATP hydrolysis and proton translocation during the operation of an E_1E_2-type H^+-ATPase is far from complete (Briskin, 1990; Briskin & Hanson, 1992). A variable rather than a fixed stoichiometry is conceivable (Stein, 1990). Nagle and Nagle-Tristram (1983) are more explicit if they state that "the vectoriality of the H^+ translocation

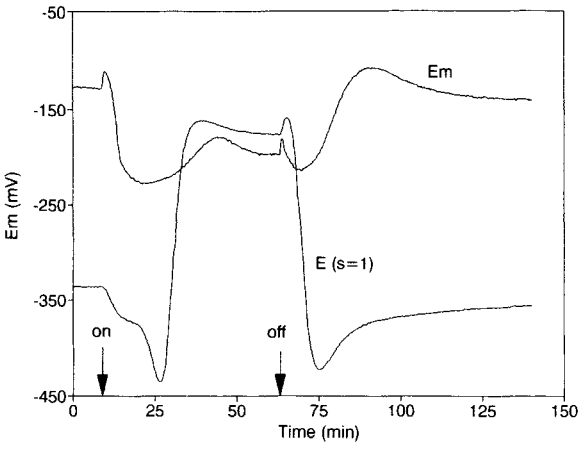

Fig. 6. A comparison between the measured E_m and the simulated E when assuming a *fixed* pump stoichiometry of 1. Arrows refer to light-on and light-off.

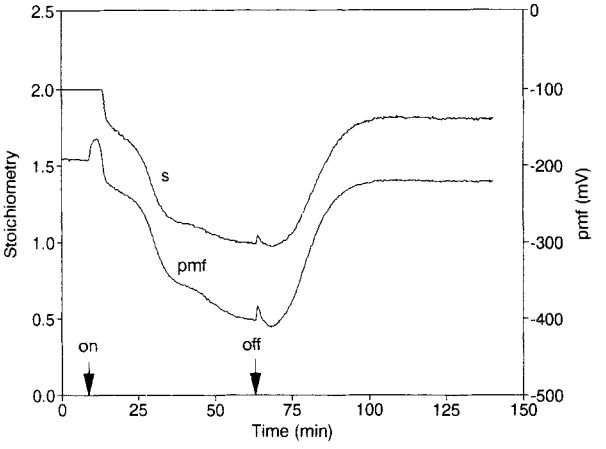

Fig. 7. Relation between the proton motive force (pmf) at the lower leaf side (defined as $\mu_{H^+,in} - \mu_{H^+,out}$) and the calculated H^+/ATP stoichiometry (s) of the proton pump. The maximum stoichiometry was assumed to be two. Arrows refer to light-on and light-off.

is a statistical concept." Consequently, as the opposing transmembrane electrochemical potential builds up, the stoichiometry will decrease. A variable H^+/ATP-stoichiometry was also suggested for the gastric H^+/K^+-ATPase (Rabon & Reuben, 1990) and for the renal Na^+/K^+-ATPase (Goldshlegger, Shahak & Karlish, 1989), both belonging to the same family of E_1E_2-ATPases. A variable stoichiometry requires an indirect coupling between ATP hydrolysis and the translocation of H^+. Such an indirect coupling was suggested by the differential effects of DCCD on these two parameters (Hsu, Rodenbach & Tu, 1992).

The recorded data of the *Potamogeton* E_m did not fit with a stoichiometry of one: the actual E_m is much less hyperpolarized (Fig.6). On the other hand, under steady-state polar conditions the calculated pmf at the lower leaf side (−400 mV, inside negative, Fig. 3*C*) is not in accordance with a pump operating with a stoichiometry of two. For that reason, the present model is based on a variable stoichiometry and the value of s was related to the calculated pmf. Concurrently with the acidification at the lower leaf side, there is a shift in the direction to the translocation of one H^+ per hydrolyzed ATP. The time courses of pmf and s are shown in Fig. 7. As a consequence of the calculated value of $\Delta G_{ATP}/F$ (−400 mV), s did not become smaller than one. This supports experimental evidence as pump stoichiometries exceeding the range of 1–2 were not (yet) reported. For the same reason, the maximum value of s was assumed to be two. The obtained value of E_p was, in turn, used to calculate E. The authors are aware that this procedure contains an element of circular reasoning: the recorded E_m data were used to simulate E_m. Alternatively, s could be calculated from the simulated E rather than from the experimentally obtained E_m. We calculated E in this way by using an iterative procedure. The calculation produced values for E somewhat different from the ones shown in Fig. 5. The oscillating pattern was nearly identical but the fit with the actual data was not as good (*result not shown*).

Properties of the Model

In the model, the trigger for the membrane hyperpolarization after light-on is the alkalinizing CO_2 uptake. Thus, a change in external pH hyperpolarizes E. An advantage of our approach is that the model accounts for the activity of the pump in the dark. This dark activity of the H^+-ATPase is omitted very often in models describing the effect of light on E_m (Cruz-Mireles & Ortega-Blake, 1991).

On the other hand, the actual situation is of course far more complex, light may induce direct or indirect, photosynthesis-mediated, changes in the membrane conductance (G_m) or in pump activity, thus in G_p. In *Nitella*, Vredenberg and Tonk (1973) found short and long-term effects of light, representing an increase of G_m and an activation of the proton pump, respectively. The light-induced changes in these two parameters were supposed to be responsible for the initial depolarization and subsequent hyperpolarization of E_m. A change in cytosolic reducing equivalents (Elzenga & Prins, 1987), a phosphorylation of the transport protein (Budde & Randall, 1990; Linnemeyer, Van Volkenburgh & Cleland, 1990) or the level of cytoplasmic inorganic

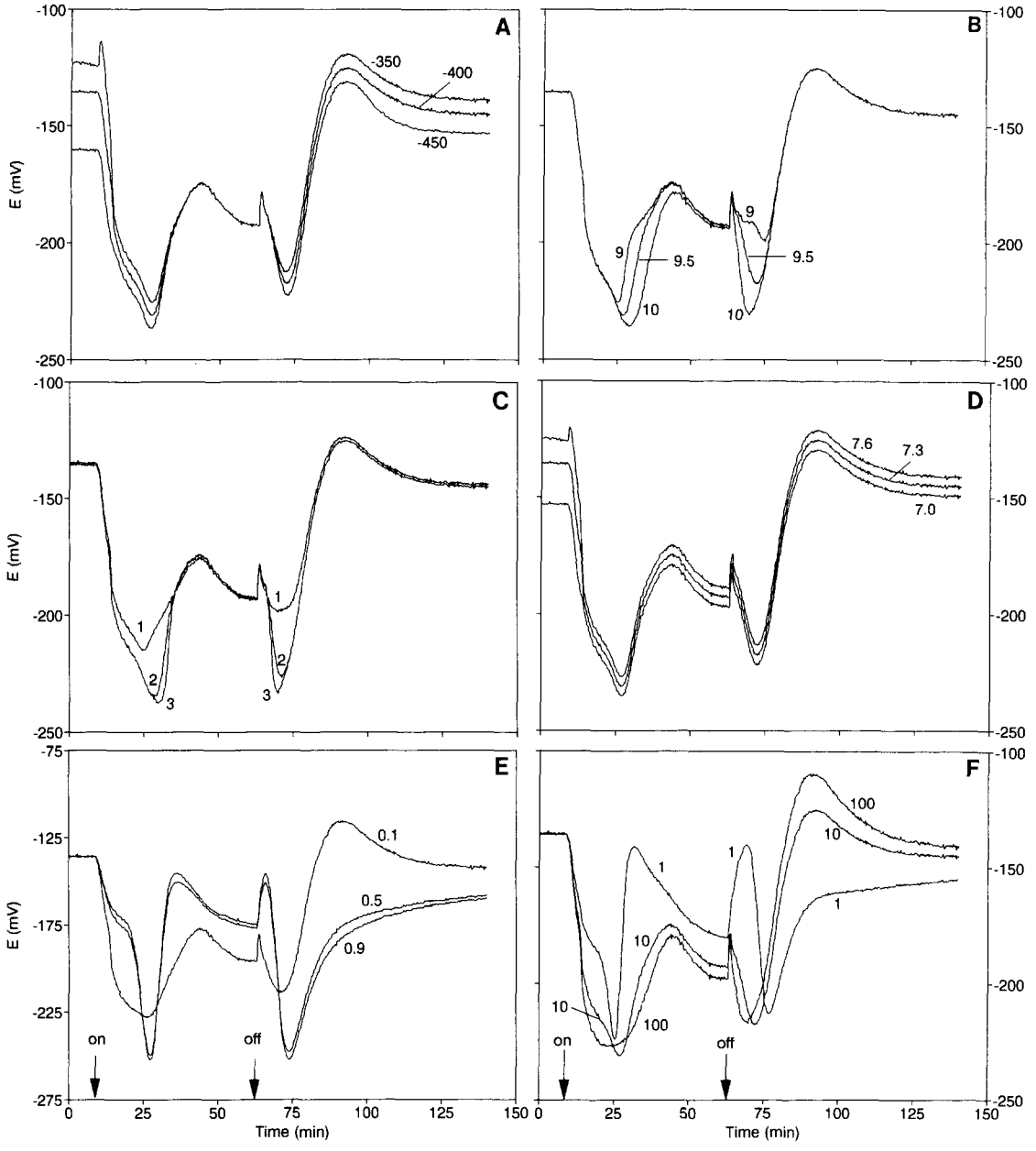

Fig. 8. Effect of changing the parameter values on the simulated membrane potential. (*A*) Effect of $\Delta G_{ATP}/F$; (*B*) Effect of pK; (*C*) Effect of number of protonation groups (n); (*D*) Effect of cytoplasmic pH; (*E*) Effect of fraction of transporters located at the upper leaf side (α); and (*F*) Effect of the ratio of the channel and pump conductance of a *single* transporter (β). Arrows refer to light-on and light-off.

phosphate (Takeshige et al., 1992) are, for instance, possible intermediates in the signal transducing pathway. Alternatively, pH_{cyt} may be involved in the regulation of G_p (Beffagna & Romani, 1991). In the model, such a change in G_p could be expressed by changing the values of some parameters after the dark-light transition. However, a sudden switch in the parameter values resulted in a sudden change of E (*result not shown*). On the other hand, more gradual changes would require specific relations between these parameters and the external pH. Therefore, all the parameters were assumed to be light independent. This is, most probably, the reason why the simulation did not account for the observed initial depolarization after light-on, indicating that part of the mechanism may indeed be light-triggered changes of G_m or G_p. The hypothesized interrelation between s and pmf resulted in a gradual decline of

s. The interpretation may be that the oscillations of E_m can be simulated by a light-induced change in s, shifting its value from two in the dark to one in the light. Alternatively, two populations of H^+-ATPases with a stoichiometry of either two or one can be imagined. A shift in the relative contribution of each may change the overall stoichiometry (Lucas, 1982).

It was expected that when E is dominated by $(E_{H^+})_{up}$ (phase 3) small variations in those parameters, which only affect E_p or G_p, should hardly have any effect on E. Thus, during the third phase, E should be almost insensitive to small variations in $\Delta G_{ATP}/F$, as the influence of this parameter is restricted to the value of E_p. On the other hand, when E is determined by E_p (phase 5), variations in pK should have a minor effect on E as the value of pK is high compared with the leaf surface pH in the dark. To test this, the value of one parameter was varied, while all the others remained constant and adopted the value used in Fig. 5. The results shown in Fig. 8 support these predictions. At the end of the polar phase (phase 3), when the value of E is determined by $(E_{H^+})_{up}$, E was indeed insensitive to variations in $\Delta G_{ATP}/F$ (Fig. 8*A*). On the contrary, in the dark (phase 5) E was unaffected by a change of pK (Fig. 8*B*). However, the steady-state value during the third phase was also insensitive to the value of pK, apparently due to the high pH and the strong increase of G_{H^+} at the upper leaf surface.

The significance of the value of n for E is depicted in Fig. 8*C*: differences between values of n of one, two or three were remarkable. A value of one damped the oscillation, whereas a value of three induced a slight amplification of the response compared with the response observed at a value of two. Such behavior was expected as the value of n determines the energy levels between the two states (protonated *vs.* deprotonated). Considering the temperature dependency of pK, in Eq. (6), a Boltzmann distribution is recognized. In that respect, the expression is similar to the one which describes the fraction of open to closed voltage-sensitive ion channels. Mathematically, the number of protonation steps is equivalent to the gating charge used for these voltage-gated channels, as both terms determine the steepness of the distribution function. The steady-state values of E in the light as well as the dark were insensitive to changes of n.

The effect of pH_{cyt} is more difficult to predict as this parameter has an effect on E_p as well as on E_{H^+}. Variations of 0.3 pH units resulted in a more or less parallel shift of the entire curve (Fig. 8*D*). It is expected that in the dark the value of α hardly has an effect on E, as the pH's on both sides are equal and E is independent of α. Such an effect was, indeed, found. However, at high values of α, E became independent of α in the light as well. (Fig. 8*E*). The effect of β is shown in Fig. 8*F*. As was seen at high values of α, assuming a value of $\beta = 1$ resulted in a strong deviation from the experimentally observed E_m. Apparently, it has to be assumed that most of the transporters are located at the lower side of the leaf and that the channel conductance of a single transporter is significantly higher than the pump conductance.

The present model, undoubtedly, oversimplifies the phenomenon of the response of the *Potamogeton* membrane potential to dark-light transitions—for instance with respect to the constancy of some parameters under different light conditions. It is fully realized that, to obtain results which fit the actual measured data more precisely, the model will have to be extended to include other parameters as well. Therefore, the model must be considered as a kind of a minimum model. Nevertheless, we demonstrated that the postulated H^+ translocating complex can account for some features of the light-induced oscillations of the membrane potential as observed in *Potamogeton* leaf cells. The model indicates a varying H^+/ATP stoichiometry of the proton pump: after light-on, at the acidifying lower leaf side the stoichiometry gradually decreases from a value of two to one.

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Appendix I

Compared with *Potamogeton* and *Elodea*, E_m in other aquatic plants is usually more sensitive to the external K^+ concentration. To account for a K^+ conductance (G_{K^+}) as well, the equivalent circuit of Fig. 2 and Eq. (9) has to be adjusted. The extended equivalent circuit is depicted in Fig. 9. It is assumed that G_{K^+} is equal on both surfaces of the leaf.

Then E is given by:

$$G_{K^+}(E - E_{K^+}) = \frac{\alpha^2\left(E_p - E + (E_{H^+} - E)\frac{G_{H^+}}{G_P}\right)_{up}}{\left(1 + \frac{G_{H^+}}{G_p}\right)_{up}} + \frac{(1-\alpha)^2\left(E_p - E + (E_{H^+} - E)\frac{G_{H^+}}{G_p}\right)_{low}}{\beta\left(1 + \frac{G_{H^+}}{G_p}\right)_{low}} \quad (12)$$

As can be seen, the incorporation of the K^+ conductance results in an implicit formulation of E, in contrast to Eq. (9), in which E is expressed explicitly in terms of H^+. Consequently, the value of E has to be found by an iterative procedure. Secondly, the possibility of more than one solution may arise. Another consequence is that an expression for the absolute value of G_{K^+} is required.

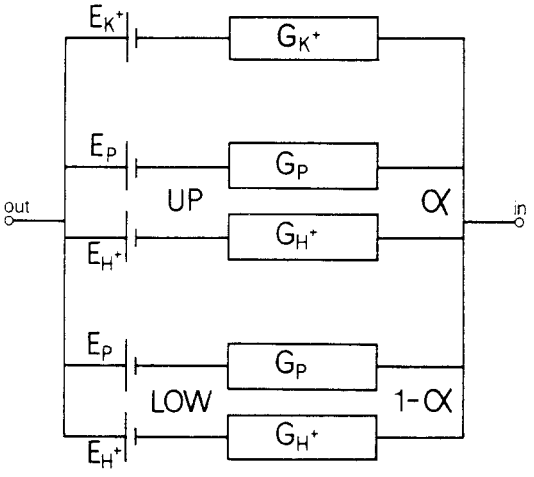

Fig. 9. The equivalent circuit of Fig. 2 extended with an equal potassium conductance (G_{K^+}) at either side of the leaf.