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Effect of High pH on the Plasma Membrane Potential and Conductance in *Elodea densa*

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Summary. In leaves of *Elodea densa* the membrane potential measured in light equals the equilibrium potential of H^+ on the morphological upper plasma membrane. The apoplastic pH on the upper side of the leaf is as high as 10.5–11.0, which indicates that alkaline pH induces an increased H^+ permeability of the plasmalemma. To study this hypothesis in more detail we investigated the changes in membrane potential and conductance in response to alterations in the external pH from 7 (=control) to 9 or 11 under both light and dark conditions.

Departing from the control pH 7 condition, in light and in dark the application of pH 9 resulted in a depolarization of the membrane potential to the Nernst potential of H^+. In the light but not in the dark, this depolarization was followed by a repolarization to about −160 mV. The change to pH 9 induced, in light as well as in dark, an increase in membrane conductance.

The application of pH 11, which caused a momentary hyper- or depolarization depending on the value at the time pH 11 was applied, brought the membrane potential to around −160 mV. The membrane conductance also increased, in comparison to its value at pH 7, as a result of the application of pH 11, irrespective of the light conditions.

Key Words plasma membrane · H^+ permeability · membrane potential · membrane conductance

Introduction

In light, the submerged leaves of the macrophytes *Potamogeton* and *Elodea* acidify the apoplast and the unstirred layer of the lower epidermis. Under natural conditions, i.e., in alkaline bicarbonate-containing waters, the resulting conversion of bicarbonate into CO_2 ensures the CO_2 supply for photosynthesis. The acidification at the morphological lower side of the leaf is accompanied by an alkalinization at the upper epidermis (Prins et al., 1980, 1982; Elzenga & Prins, 1988, 1989). When the apoplastic pH of the upper epidermis and the membrane potential (E_m) are measured simultaneously, it can be calculated that the proton motive force (pmf) across the plasma membrane on the abaxial (upper) side approaches zero. At high pH, apparently the proton conductance (G_{H^+}) greatly exceeds the conductances of other ions and also that of the electrogenic proton pump (G_p) and E_m is dominated by the diffusion, i.e., equilibrium or Nernst potential of H^+ (E_{H^+}).

In a previous study it was demonstrated that a low pH near the upper plasmalemma inhibited polarity almost completely in leaves of *Potamogeton lucens*. This effect was ascribed to a strongly reduced proton permeability (P_{H^+}) of the plasma membrane, induced by the low apoplastic pH (Miedema & Prins, 1991). Such a pH dependency of P_{H^+} of the plasma membrane on the external pH has also been found in *Chara*. In *Chara*, at high pH (pH > 10.5), the so-called H^+ state is induced while, at neutral pH E_m is dominated by the proton pump and the cells are said to be in the pump state (Bisson & Walker, 1980; Bisson, 1986; Beilby, 1990).

There are numerous reports about the dependency of E_m on the external pH (Kitasato, 1968; Spanswick, 1972*b*; Richards & Hope, 1974; Felle & Bentrup, 1976; Bisson & Walker, 1980; Bisson, 1986; Takeshige, Shimmen & Tazawa, 1986; Dahse et al., 1987; Beilby, 1990; Cruz-Mireles & Ortega-Blake, 1991). Information on the kinetic response to changes in the external pH is limited, however. In order to gain more insight into these processes, the changes in membrane potential and conductance (G_m) in response to stepwise alterations in pH from 7 to 9 or 11 have been studied.

Materials and Methods

Culturing Conditions

Elodea densa were grown indoors in tanks on a clay substrate covered with a thin layer of sand. The tanks were filled with demineralized water, and the subsequent nutrient concentra-

tions were determined: $K^+ < 0.1$ mM; $Na^+ < 0.1$ mM; $Ca^{2+} = 0.5$–1.3 mM; and $Cl^- < 0.1$ mM. The pH varied between 7.5 and 8.5, and the temperature was 21°C. The lighting regime was 12 hr light alternated with 12 hr dark. The high pressure mercury lamps (Philips, HPLN 400W) radiated only minimal UV rays. The light intensity at the water surface was 90 μmol/m^2 · sec.

Experimental Conditions

The experimental standard and control solution contained (in mM): 4.5 $CaCl_2$, 2.5 KCl and 0.5 $KHCO_3$ with a final pH of 7. For some of the electrophysiological experiments *Elodea* twigs were kept in the standard solution under a 12 hr light/12 hr dark regime for three weeks. During this period the absolute value of E_m did not change, neither did the light/dark response of E_m. Where appropriate, the standard solution was supplemented with 50 mM MES/Tris, 50 mM TAPS or 50 mM CAPS and adjusted with KOH to pH 7, 9 or 11, respectively.

A small leaf strip was mounted in a continuous flow-through 1-ml vessel. The flow velocity was about 10 ml/min. The light intensity, measured as total radiation, was 100 μmol/m^2 · sec. The experiments were performed at an ambient temperature of 20°C.

Electrophysiology

For the electrophysiological measurements, standard 3-M KCl-filled microelectrodes were used. E_m was not corrected for the tip potential (about −10 mV). It is unknown which cell type, from the upper or from the lower cell layer, is impaled by the microelectrode. Preliminary experiments, however, did not reveal any difference in E_m when the electrode was inserted in either the upper or lower cell layer (H.B.A. Prins, *unpublished results*).

Conductance measurements were done using the two-electrode method (Tretyn, Wagner & Felle, 1991). One electrode was used for current injection, the other for recording E_m and any changes thereof caused by the current injection. Due to the small size of the cells it is almost impossible to impale two electrodes into a single cell. Therefore, the second microelectrode was inserted in another cell at a distance of about 125 μm, i.e., about 10 cells away from the cell which was impaled by the first electrode. On the current electrode an alternating hyper- or depolarizing square current of 1 nA and of 100-msec duration was superimposed every 2 sec. As different cells are impaled, changes in the plasmodesmatal resistance may interfere with the recorded signal of the voltage electrode. As already mentioned above, both electrodes showed an identical response of E_m. Moreover, it was already demonstrated that the *Elodea* cells are interconnected by numerous plasmodesmata (Goodwin, Shepherd & Erwee, 1990). The presence of this intercellular symplast can explain the observed high electric coupling between the *Elodea* cells (Spanswick, 1972*a*). Therefore, it was assumed that the plasmodesmatal resistence was low compared to the resistance of the plasma membrane. Then, changes in the signal response of the voltage electrode do reflect changes of G_m rather than changes of the plasmadesmatal conductance. This technique renders only qualitative information about the value of G_m. A relatively large perturbation of E_m indicates a relatively small value of G_m.

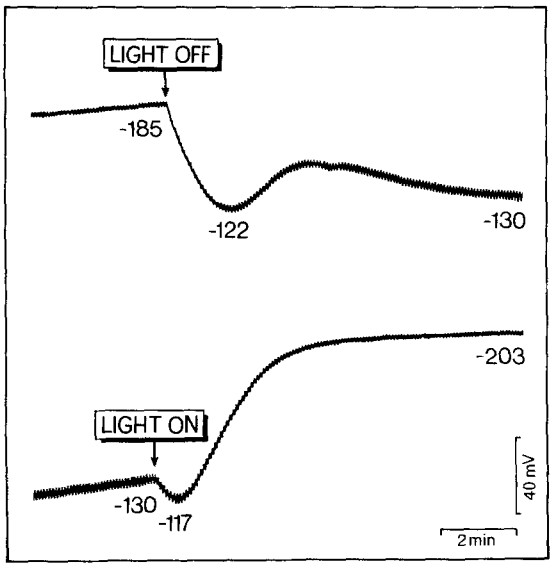

Fig. 1. The dark and light responses of the membrane potential (E_m) and conductance (G_m) of the *Elodea* cell. The unbuffered solution consisted of the control solution containing (in mM): 4.5 $CaCl_2$, 2.5 KCl and 0.5 $KHCO_3$, pH 7. The superimposed signal on the voltage trace is a measure of the membrane conductance. A large perturbation indicates a low conductance.

Results

Unbuffered Solution

Figure 1 shows the dark and light responses of E_m in unbuffered solution pH 7. The steady-state value of E_m in the light was −185 mV. Light off induced, after a slight oscillation, a depolarization of 55 mV which resulted in a dark level of −130 mV. G_m decreased in the dark. When the light was switched on again, E_m initially depolarized to −117 mV but subsequently hyperpolarized to −203 mV; this was slightly more negative than the initial level in the light. G_m increased again in the light.

The effect of the buffer capacity of the medium on E_m at pH 7 was nil; switching from the unbuffered solution to a 50-mM MES/Tris-buffer pH 7 did not change E_m significantly (*result not shown*).

Effect of K^+ Concentration

To fix the apoplastic pH on a value as high as 9 or 11, it proved to be necessary to use strong buffered solutions. The standard solution was supplemented

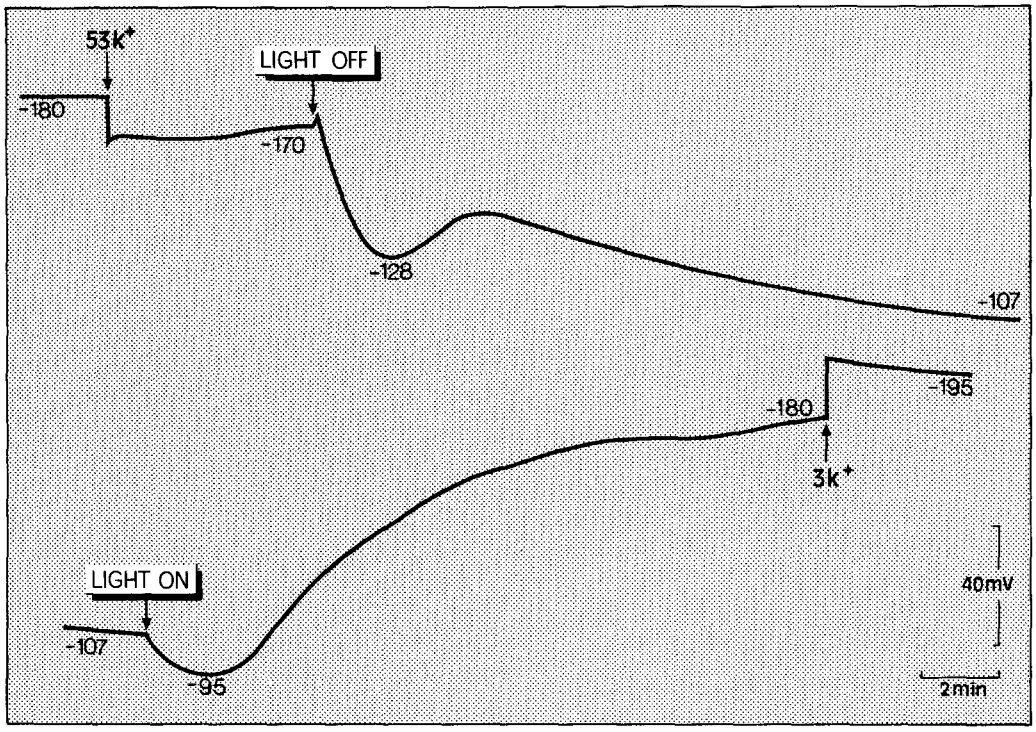

Fig. 2. The effect of a low (3 mM) and a high (53 mM) K^+ concentration on E_m, as well as the dark and light response in the high K^+ solution.

either with 50 mM TAPS or with 50 mM CAPS buffer and brought to the desired pH. The pH adjustment with KOH resulted in an increase in the K^+ concentration up to 33 mM at pH 9 and up to 53 mM at pH 11. Therefore, the effect of high external K^+ was studied. The high K^+ medium consisted of the (unbuffered) standard solution supplemented with 50 mM KCl which resulted in a final K^+ concentration of 53 mM.

The effect of this K^+ concentration on E_m in light and dark is shown in Fig. 2. Switching, under lighted condition, from the control (3 mM) to the high (53 mM) K^+ solution depolarized E_m almost instantaneously from -180 to -170 mV. The subsequent response to light off under the high K^+ regime was, with the exception of a very small initial hyperpolarization that was not observed in the control solution, otherwise quite similar to the one observed in the control. In the dark, E_m gradually depolarized and reached a final value of -107 mV, i.e., 63 mV less negative than the value with light on and 23 mV less negative than the dark level at low K^+ shown in Fig. 1. Light on induced a response similar to the one observed at low K^+: after an initial depolarization a subsequent hyperpolarization to -180 mV was observed. Lowering the external K^+ concentration to the control level led to an almost instantaneous hyperpolarization. After a slow decline a final value around -195 mV was reached.

Effect of pH 9

The responses of E_m and G_m to the change from pH 7 to 9 are shown in Fig. 3. In the light the increase in pH resulted in a rapid depolarization of E_m from -185 to -92 mV followed by a slow partial repolarization to -160 mV (Fig. 3*A*). The change to pH 9 induced an increase of G_m. The response of E_m was reversible. Changing back to pH 7 resulted in a fast hyperpolarization and finally a steady state near to the original level. G_m also increased again, although it did not reach its original level.

In the dark, the switch from pH 7 to 9 caused E_m to depolarize from -128 mV to a steady level at -90 mV (Fig. 3*B*), the same level that was observed during the initial transient depolarization in the light. A repolarization of E_m, as observed in the light, did not occur. The response was reversible but the repolarization after washing with pH 7 was much slower in the dark than in the light. As in the light, pH 9 in the dark induced a reversible increase of G_m.

Effect of pH 11

The response of E_m to pH 11 is shown in Fig. 4. In these experiments, buffers of different composition were used. The solutions were adjusted to pH 11

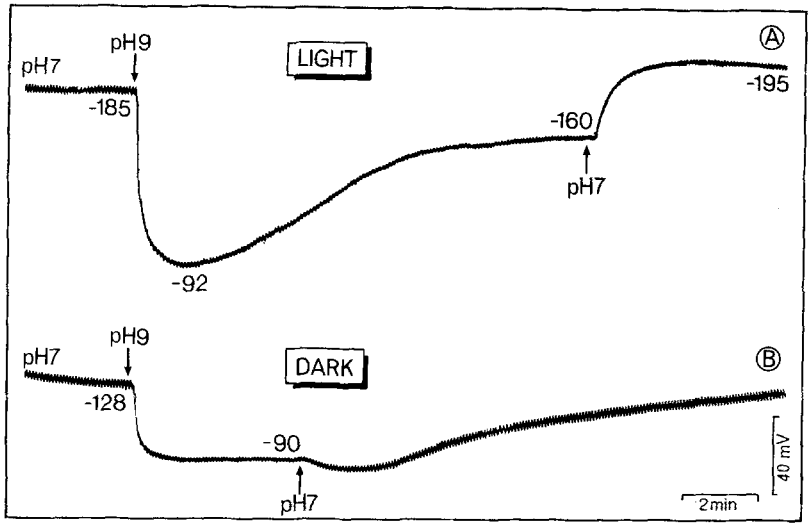

Fig. 3. The reaction of E_m and G_m to a switch from pH 7 to 9 and turning back again to pH 7 in the light (*A*) and in the dark (*B*). The buffers consisted of the standard solution supplemented with 50 mM MES/Tris (pH 7) or with 50 mM TAPS (pH 9) and adjusted to the desired pH with KOH.

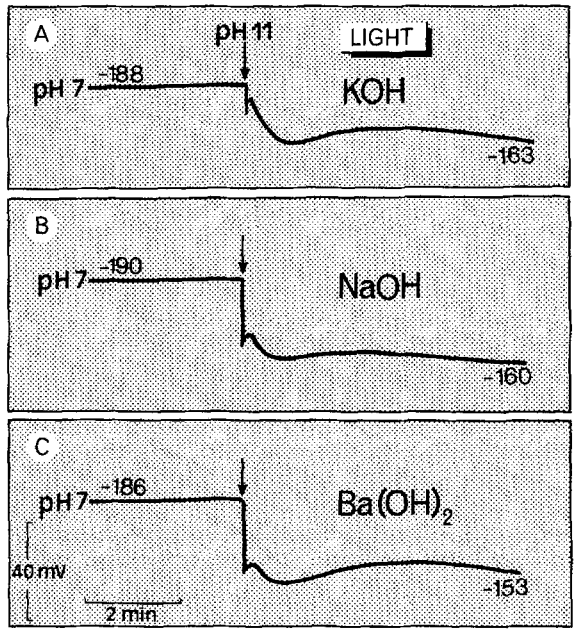

Fig. 4. The response of E_m after switching from the pH 7 buffer to three different pH 11 buffers. The 50-mM CAPS buffers were adjusted to pH 11 using KOH (*A*), NaOH (*B*) or $Ba(OH)_2$ (*C*).

with KOH (Fig. 4*A*), NaOH (Fig. 4*B*) or $Ba(OH)_2$ (Fig. 4*C*). In all traces an almost instantaneous depolarization of E_m was observed. After a slight oscillation E_m stabilized at about -160 mV, a value about 28 mV less negative than the value at pH 7. The similarity of the responses provides additional evidence for the low sensitivity of E_m to external K^+.

The effect of pH 11 was reversible as is shown in Fig. 5. In the light, returning to pH 7 caused a temporary depolarization of E_m to -107 mV followed by a repolarization to -190 mV (Fig. 5*A*). As observed in the case of pH 9, pH 11 also induced a reversible increase of G_m.

Figure 5*B* shows the response to the change from pH 7 to 11 in the dark: a fast hyperpolarization of E_m from -122 to -175 mV. As found in the light, returning to pH 7 initially led to a depolarization of E_m, in this particular experiment to -50 mV. After an extended period E_m always repolarized to the control dark level (around -130 mV). Application of pH 11 when E_m was still depolarized led to an almost instantaneous hyperpolarization of E_m from -67 to -170 mV which was followed by a slow decline to -162 mV (Fig. 5*C*).

As found in the light, pH 11 also induced in the dark a reversible increase of G_m.

The Table summarizes the dependency of E_m to the external pH. For comparison the value of E_m at pH 5.5 in the light is also mentioned.

Discussion

Assuming a cytosolic K^+ concentration of about 174 mM (Dahse et al., 1987) it is obvious that under the high K^+ regime (Fig. 2) E_m is not determined by the diffusion potential of K^+. This, apparently, is also true at high external pH as is elegantly demonstrated by the response shown in Fig. 5*C*. Despite the jump in external K^+ (from 3 to 53 mM), the change from pH 7 to 11 effected an almost immediate hyperpolarization of more than 100 mV.

The low sensitivity of E_m to high external K^+ concentrations is remarkable in view of observations on other aquatic plants. In the range of 1 to 100 mM K^+, E_m in *Nitella* exhibits a sensitivity of 59 mV

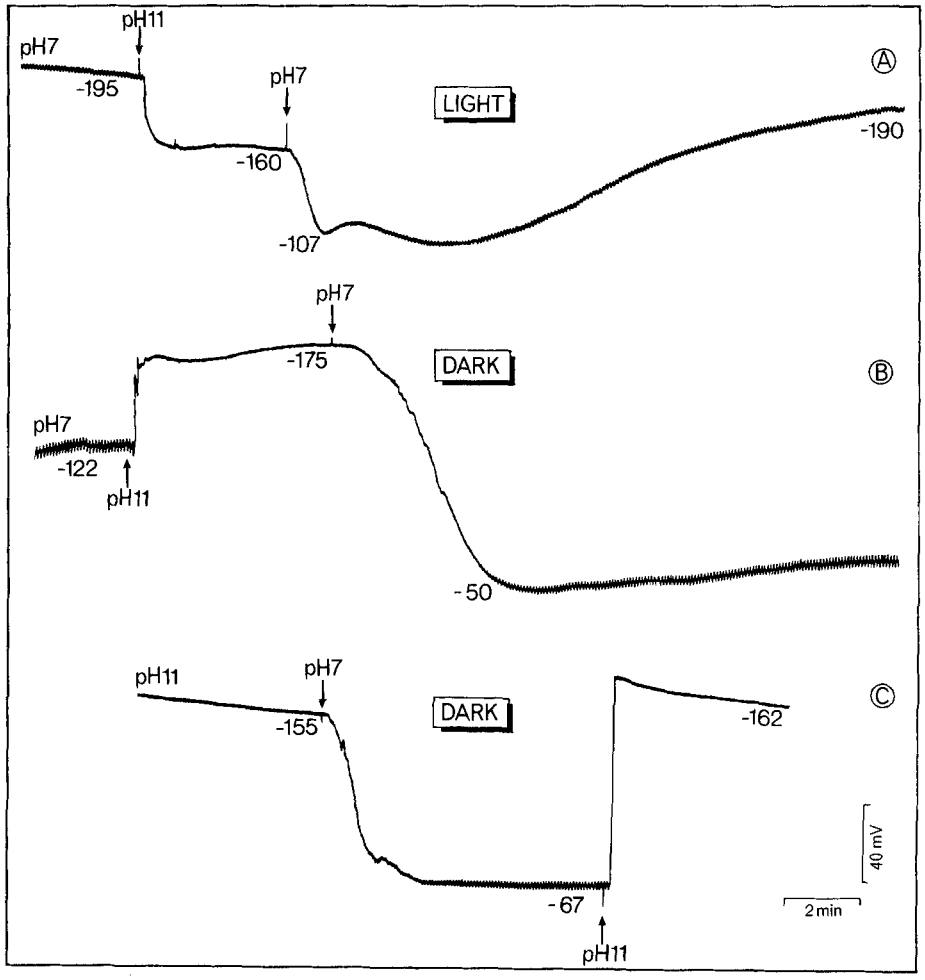

Fig. 5. The effect of changing from a 50-mM MES/Tris buffer pH 7 to a 50-mM CAPS buffer pH 11 and vice versa on E_m and G_m under light (*A*) or dark (*B* and *C*) conditions.

Table. The value of E_m and the standard error (in mV) in relation to the external pH, under dark and light conditions[a]

pH	Dark	Light
5.5	—	−156 ± 8 (3)
7.0	−132 ± 4 (11)	−189 ± 7 (13)
9.0	−94 ± 5 (4)	−163 ± 5 (5)
		transient −96 ± 4 (5)
11.0	−160 ± 3 (4)	−161 ± 5 (5)

[a] The 'transient' mentioned at pH 9 in the light, refers to the observed initial transient depolarization of E_m after changing from pH 7 to 9. The number in parentheses represents the number of experiments.

per decade, indicating that the plasma membrane behaves as a K^+ electrode (Cruz-Mireles & Ortega-Blake, 1991). At K^+ concentrations above 1 mM, the *Chara* cell is also forced into the so-called K^+ state (Beilby, 1990). Because of the low K^+ conductance E_m of *Elodea* can be described almost solely in terms of H^+. Then, the value of E_m is determined by the plasmalemma-bound electrogenic H^+ ATPase in the lower cell layer and by the passive H^+ conductance in the upper.

The results of the present experiments are in some respect very similar to those obtained by Bisson et al. with *Chara* (Bisson & Waker, 1980; Bisson, 1986). In the *Chara* cell, at high external pH, G_{H^+} greatly exceeds all other conductances. The *Elodea* plasmalemma of the upper cell layer behaves similarly. Consequently, the *Elodea* E_m is fixed at E_{H^+}, irrespective of the electrogenic H^+ extrusion at the lower side of the leaf.

A significant increase of G_{H^+} at an external pH of 9 and a cytoplasmic pH of 7.3 (as indicated by preliminary experiments using intracellular pH electrodes) would cause E_m to depolarize to circa −100 mV, inside negative. It seems, therefore, that the depolarization to −92 mV as seen in Fig. 3*A*, reflects the transition from the pump state at pH 7 to the H^+ state at pH 9. E_m is temporarily short circuited at

E_{H^+} due to a sudden increase of G_{H^+}, as is reflected in the observed increase of G_m. An indication for the increase of P_{H^+} compared to P_{K^+} may come from the calculated ratio of these two permeability coefficients. Assuming that the value of −92 mV reflects a diffusion potential composed of H^+ and K^+, P_{H^+}/P_{K^+} appeared to be around 10^8.

Apparently, a subsequent shift in G_p/G_{H^+} partly repolarized E_m to a steady-state level of −160 mV. During this phase, G_m hardly changed. This may indicate that the increase of G_p was balanced by a decrease of G_{H^+}.

In the dark, pH 9 caused a depolarization to the same level (−90 mV) that was observed in the light. This suggests that also in the dark pH 9 induced an increase of G_{H^+}, which is in accordance with the observed increase of G_m. The absence of the subsequent repolarization, which did occur in the light, probably was a result of decreased pump activity. The latter would also explain the much longer time needed in the dark to repolarize E_m after changing back to pH 7.

Assuming that the immediate response at pH 9 was effected by the high external pH, it would be quite logical to expect an even quicker response at pH 11, as then G_{H^+} increases even further and E_{H^+} becomes more negative (−215 mV inside negative at an internal pH of 7.3). While G_m increased, the reaction of E_m to pH 11 was, indeed, almost instantaneous, which indicates a immediate decrease in G_p/G_{H^+} by an increase of G_{H^+}. Now, the calculated P_{H^+}/P_{K^+} was about 10^9, thus slightly higher than the value during the transient in pH 9 in the light. A decrease in G_p *alone* would be an alternative explanation for the depolarization of E_m at pH 11 in the light (Fig. 5*A*). This, though, would make it difficult to explain the observed increase of G_m. However, both the hyperpolarization of E_m and the increase of G_m in response to pH 11 in the dark (Fig. 5*B* and *C*) would be in accordance with an increase of G_p, the more as the electromotive force of the pump (E_p) will become more negative at an increasing external pH. Although an effect on G_p cannot be excluded, we believe that the very fast responses suggest that the effects are on G_{H^+} rather than on G_p.

It is significant that, in all cases, E_m was less negative than the predicted −215 mV. The most reasonable explanation is that the pH close to the outer membrane deviated, despite the high buffer concentration of 50 mM, from the pH of the bulk phase. This would cause E_{H^+} to be less negative than calculated. An apoplastic pH of just 10.4 would induce a response as shown in Fig. 5*A*: a depolarization of E_m instead of a hyperpolarization. Alternatively, the cytosolic pH might be increased by the high external pH, as was found in *Chara* (Reid & Smith, 1988). An alkalinization of the cytoplasm would depolarize E_{H^+} and could (partly) explain the discrepancy between the recorded and the calculated value.

In the light, as well as in the dark, the response to pH 11 was reversible. Under both conditions, washing with pH 7 induced a depolarization (Fig. 5). Apparently, G_{H^+} initially remained high during the switch to pH 7. Consequently, an influx of H^+ due to an increased (i.e., less negative) E_{H^+}, depolarized E_m. A shift in G_p/G_{H^+} subsequently caused a slow repolarization of E_m to −185 mV, a level indicating that the cell had returned to the pump state. The difference in time needed to repolarize E_m after returning to pH 7 in light and dark may reflect a decreased pump activity under dark conditions.

Conclusions

Under light and dark conditions the plasmalemma of *Elodea* exhibits a remarkable pH-dependent conductance to H^+. Switching from pH 7 to 11 resulted in an almost instantaneous change in membrane potential. The present data can be interpreted as evidence for the existence of H^+-conducting channels in the plasma membrane of the upper epidermal cells. Apparently, these putative H^+ channels are regulated by the external pH. The changes in membrane potential and conductance induced by alkaline pH were reversible. However, the changes induced by an increase in pH had a much shorter time constant than those changes from high to low pH.

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