Coupling of water and potassium ions in K^+ channels of the tonoplast of *Chara*

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ABSTRACT Electrokinetic measurements, of streaming potential, were carried out on an excised inside-out patch of the vacuolar membrane of *Chara* corallina. A water activity gradient was imposed across the patch membrane containing a single K^+ channel by addition of sorbitol to one side. Two different K^+ channels were found in the tonoplast. Their open channel conductance was investigated as a function of KCl concentration. They had a maximal open channel conductance of 247 and 173 pS, and an apparent affinity (K_M) of 116 and 92 mM, respectively. Single-channel zero-current potentials were determined in the presence of an osmotic gradient, and dilution artifacts were corrected for by addition of valinomycin to the bath. Our results suggest that 29 water molecules were coupled to the transport of one K^+ ion in the large conductance K^+ channel which has a pore radius of ~1.5 nm.

INTRODUCTION

In plant cells most ions are stored in a large central vacuole which plays an important role in turgo- and osmoregulation. The tonoplast which surrounds the vacuole contains various ion pumps and ion channels (Hedrich and Schroeder, 1989). The study of the function of ion channels has mostly been concerned with the rate of ion movement through these integral membrane proteins.

Cytoplasmic droplets formed from *Chara* are covered by a membrane derived from the tonoplast (Lühring, 1986; Sakano and Tazawa, 1986). The passive conductance of this vacuolar membrane is largely dominated by a potassium conductance (Homblé, 1987).

Different K^+ channels have been found in the tonoplast surrounding cytoplasmic droplets formed from *Chara* (Homblé et al., 1987; Tyerman and Findlay, 1989; Homblé and Fuks, 1991). In symmetrical 150-mM KCl solutions, K^+ channels with open channel conductances of 170 pS (Laver et al., 1989), 130 pS (Laver et al., 1989; Homblé and Fuks, 1991), and 90 pS (Homblé and Fuks, 1991) have been identified in excised patches. The unitary current through the maxi-K channel (170 pS) is limited, at high voltage, by ion diffusion external to the pore and, at high potassium concentration, by the maximum transport rate of the channel (Laver and Walker, 1987; Laver et al., 1989). Both 170 and 130-pS K^+ channels have a high selectivity for K^+ over other monovalent alkali cations (Lühring, 1986; Laver and Walker, 1987; Bertl, 1989; Homblé and Fuks, 1991; Laver and Walker, 1991). Unfortunately, the physiological role of those K^+ channels is not yet understood.

Selective channels exclude all but those ions which they are specifically designed to conduct. Substances that cannot flow through the channel act osmotically on the channel. Inside narrow channels, ions, and water are constrained to diffuse in a single file. If a membrane containing this type of channel separates two solutions of different osmolalities, a streaming potential difference is generated (Rosenberg and Finkelstein, 1978; Levitt et al., 1978; Miller, 1982; Finkelstein, 1987; Alcayaga et al., 1989).

In this work, the streaming potential arising across 130-pS K^+ channels of the tonoplast is measured by imposing an osmotic gradient across the patched membrane. The ion-water coupling was found to be large and we show that this channel is too large to assume single file diffusion assumption.

MATERIALS AND METHODS

Protoplasmic droplets

Protoplasmic droplets were obtained by gently cutting the lower end of a turgorless internodal cell of *Chara* corallina Klein ex Willd., em, R. D. W. maintained vertically and allowing the endoplasm to flow down from the open end into a 150 mmol kg^{-1} (millimolal) KCl solution. In order to study the effect of the salt concentration on the single channel conductance, protoplasmic droplets were formed in solutions of different KCl concentration and seals were formed in symmetrical salts.

Patch-clamp

After establishing a "gigaseal" between the pipette and the membrane (Hamill et al., 1981), the patch was excised into an inside-out configuration. The control of the membrane potential and the measurement of membrane currents were performed with a Bio-Logic RK-300 (BIO-LOGIC, France) patch-clamp amplifier. Giga-ohm seals of ~10–50 GΩ were formed with pipettes (10 MΩ) made from borosilicate glass. Continuous recordings of patch currents were preliminarily stored in digital form on a video tape. The data were subsequently replayed through a low pass 5-pole Tchebicheff filter (generally, −3dB cut-off frequency at 500 Hz), and the relevant events were sampled and stored digitally on a computer (Zenith Z-200). The membrane potential difference across a patch was calculated as minus the pipette potential difference. Thus, the flow of positive charges from the bath to the pipette is defined as an outward-current and is positive.

Streaming potentials

The control current-voltage relationship for the open channel was obtained in symmetrical KCl solution. Then, an osmotic gradient was

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0006-3495/92/10/996/04 $2.00

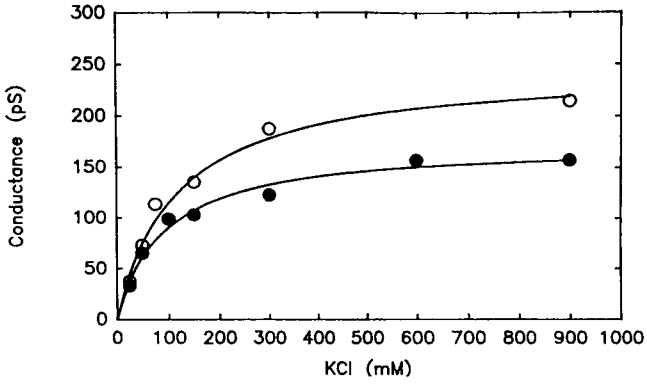

FIGURE 1 Single-channel conductance as a function of K^+ concentration for two different K^+ channels of the tonoplast of *Chara*. Measurements were done on inside-out excised-patches in symmetrical KCl concentrations. Data were fitted with the Michaelis-Menten equation. The single channel conductance was 90 pS (●) and 130 pS (○) in symmetrical 150 mM KCl.

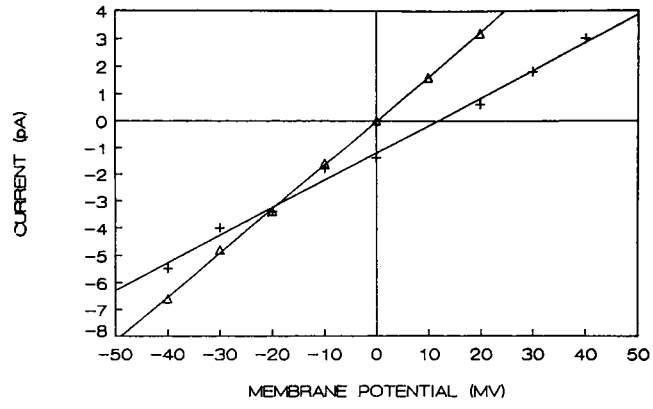

FIGURE 2 Current-voltage relationships in 300 mmolal KCl, in the absence (Δ) and in the presence of 0.65 osmol/kg sorbitol (+) on one side of the membrane. The reversal potential measured in the presence of both valinomycin and sorbitol was 1 mV.

established by changing the solution of the bath for a solution containing the same molal concentration of KCl plus 0.65 osmol kg^{-1} sorbitol. Under these conditions a second current-voltage curve was taken. Finally, 1 μM valinomycin was added in the bath and a third current-voltage curve established. This was done to correct the values of reversal potential for electric potential generated by reductions in the activity coefficient due to the presence of the sorbitol and the differences in local salt concentration in the unstirred layer (Rosenberg and Finkelstein, 1978). The streaming potential arising only from K^+-water coupling is the difference between the reversal potential for the channel measured in the presence of sorbitol and the reversal potential obtained with valinomycin. The reversal potential was determined by linear or nonlinear regression of the current-voltage relationship.

RESULTS

KCl-dependent conductance

As previously described (Homblé and Fuks, 1991), two different K^+ channels were observed in our preparations and were identified by their single channel conductance in symmetrical 150 mM KCl concentration. Many ion-selective channels exhibit current saturation with increasing permeant ion concentration reflecting occupation of one or more distinct ion binding sites within the channel. This behavior was investigated for both 130 and 90-pS K^+ channels in the presence of increasing symmetrical concentrations of potassium in order to distinguish them whatever the KCl concentration. To provide a phenomenological description of saturation behavior, the measured slope conductance at low positive or negative voltage is plotted as a function of the K^+ concentration in Fig. 1. These data were well described by the Michaelis-Menten relationship as indicated by the fitted curve. The Michaelis-Menten fit gave parameters of K_M = 116 mM and γ_{max} = 250 pS for the concentration at half-saturation and the maximal unitary conductance, respectively. The corresponding parameters for the 90-pS K^+ channel were K_M = 92 mM and γ_{max} = 170 pS.

Water coupling

The low conductance (90 pS) K^+ channel was observed only on such rare occasions (Homblé and Fuks, 1991) that electrokinetic measurements were only recorded successfully on the 130-pS K^+ channel. A streaming potential determination made in 0.65 osmol/kg sorbitol solution is shown in Fig. 2. A control current-voltage curve was taken just before application of the water activity gradient in symmetrical KCl concentrations. This curve was clearly shifted to positive values of membrane potential by decreasing the activity of water in the bath. In those conditions, the reversal potential of the current-voltage characteristic of the 130-pS K^+ channel is 10 mV (Table 1). After addition of 1 μM valinomycin the reversal potential shifted backward to plus 1 mV and the patch conductance increased a hundred times. This low value of reversal potential recorded in the presence of valinomycin suggested that the ion activity did not change significantly in the presence of sorbitol. We have confirmed this hypothesis by measuring the activity of K^+ and Cl^- by means of ion-specific electrodes. Indeed, the activity of these ions was not affected by 0.65 osmolal sorbitol (data not shown). In the presence of 0.65 osmolal sorbitol, the unitary conductance of an open 130-pS K^+ channel decreased by ~30% (Table 1).

TABLE 1 Electrical potential differences and single-channel conductance changes produced by an osmotic gradient across the 130-pS K^+ channel

	No sorbitol	Sorbitol added
V_{rev}(channel)	0 mV	9.7 + 0.8 mV
V_{rev}(channel + valinomycin)	—	0.9 + 0.1 mV
Unitary conductance	176.0 + 2.3 pS	118.3 + 5.3 pS

KCl molality (300 mmolal) was identical on both side of the tonoplast. The osmotic gradient was produced by 0.65 osmol/kg sorbitol. Data were obtained on seven replicates.

DISCUSSION

The osmotic permeability of the plasma membrane of Characean internodal cells (100 μm s^{-1}) is relatively large compared with that of most other cells (Finkelstein, 1987). For instance, the osmotic permeability of Valonia is only 2.4 μm s^{-1} (Gutknecht, 1967). The value of the osmotic permeability of the tonoplast has not yet been measured but it should be at least as large as that of the plasma membrane since the vacuole occupies 90% of the volume of mature Characean cells and both membranes are in series.

Giant internodal Characean cells, have been convenient material for the study of water relations in single cells. Using the method of transcellular osmosis Kamiya and Tazawa (1956) observed that the water movement across the plasma membrane of Nitella flexilis was polarized. The rate of water movement into the cell was 2.6 times larger than the rate of water movement out of the cell. The polarity of water movement was altered after a treatment with cytochalasins (Wayne and Tazawa, 1988). Treating the cells of Nitellopsis obtusa with different inhibitors of protein function, Wayne and Tazawa (1990) have shown that sulfhydryl reagents inhibit exosmosis, whereas nonyltriethylammonium (a K^+ channel blocker) inhibits endosmosis. Combining the voltage-clamp technique and the pressure probe technique, Kourie and Findlay (1991) observed that the K^+ conductance of the plasma membrane of *Chara* inflata increased as the turgor is decreased or the external osmotic pressure increased. Taken together, these results suggested that ion channels and water transport are coupled in the plasma membrane of Characean cells.

The shift of reversal potential observed in the presence of an osmotic gradient indicated that the water flow through the K^+ channels drives a K^+ current. The streaming potential of the 130-pS K^+ channel was 9 mV for 0.65 osmol per kg sorbitol.

Rosenberg and Finkelstein (1978) and Levitt et al. (1978) have shown that it is possible to calculate the number of water molecules that are coupled to each ion transported across the narrow region of the channel. This theory is only valid when ions and water molecules are constrained to diffuse in single file. However, there has recently been published a new theory of the streaming potential that is valid for small ion selective channels where the single file assumption is not required a priori (Levitt, 1990). According to this theory the volume of water per ion (Vol) flowing through a univalent cation-selective channel is given by:

$$Vol = -e\left(\frac{\Delta\psi}{\Delta P}\right)_{I=0},$$

where $\Delta\psi$ is the streaming potential, ΔP the pressure difference across the membrane, and e is the electrical charge.

Using the value for the streaming potential obtained for 300 millimolal KCl concentration, we can calculate that there are at least 29 water molecules which are coupled to the transport of one K^+ ion in the 130-pS K^+ channel. Such a large value of ion-water coupling does not seem unreasonable in the case of giant plant cells of *Chara*. Indeed, previous electroosmotic measurements done on an intact cell have led Barry and Hope (1969) to conclude that up to 30 water molecules move across the plasmalemma with each equivalent monovalent cation.

Levitt (1990) also showed that the streaming potential depends only on the geometry of the channel. For spherical particules of radius a in a uniform cylindrical channel of radius R and length L the volume of water molecules moved per ion is given by:

$$Vol = \frac{3\pi R^2 a}{4}\,\frac{1 - 1.33\,\alpha^2}{h + (a/L)1 - 2\,\alpha^2\,(1 - 0.67\,\alpha^2)},$$

where $h = 1 - 2.1054\,\alpha + 2.0805\,\alpha^3$, $\alpha = a/R$ and $a = 0.133$ nm.

Using this equation, our results would correspond to a pore of $\sim$1.5 nm in radius, which is not an unreasonable result for the large conductance channel considered here. Note that this value is independent of pore length because the second term in the denominator is negligible for $L > 2$ nm. This result indicates that the 130-pS K^+ channel can accommodate more than one ion which can get past each other.

The relationship between concentration and open channel conductance is nonlinear and shows a saturation which is well described by the Michaelis-Menten equation. This indicates that there is at least one ion binding site in the pore.

The unitary conductance of the 130-pS K^+ channel decreases in the presence of sorbitol (Fig. 2). This has also been observed for the Maxi-K channel in the presence of impermeant solutes such as sucrose or urea (Laver et al., 1989). According to these authors, this effect arises from a reduction of the diffusion of K^+ from the bulk aqueous phase to the pore mouth. If the access diffusion is the only factor affecting the change in unitary conductance of the channel, then one might expect that this effect will be reduced by increasing the salt concentration. Indeed, the diffusion limitation of ion flow to the channel entrance is lowered when the ion concentration is increased (Fig. 1). Our electrokinetic measurements were done using a KCl concentration which saturates the current through the channel. At this concentration the rate of ion transport becomes limited by the binding-unbinding step but not by the rate of diffusion toward the entrance of the channel. Thus, the decrease of the unitary conductance of the 130-pS K^+ channels observed after addition of sorbitol suggests that sorbitol may act directly on the pore. This conclusion is also supported by the results showing that the reversal potential measured in the absence of sorbitol and in the presence

of both sorbitol and valinomycin was not significantly different, indicating that the electrochemical potential of the K^+ ions were equal on both side of the membrane during the course of an electrokinetic experiment.

In conclusion, our results indicate that there is a strong coupling between water and K^+ in the 130-pS K^+ channel of the tonoplast of *Chara,* and we have calculated that the channel has a radius of 15 nm.

We are greatly indebted to Dr. Jack Dainty, Dr. Alan Finkelstein, Dr. Claude Grignon, and Dr. Jean-Baptiste Thibaud for their helpful comments about this manuscript.

F. Homblé is a Research Associate of the National Fund for Scientific Research (Belgium) which is kindly acknowledged for its financial support. A. A. Véry is the recipient of an Erasmus grant from the European Economic Community.

Received for publication 13 December 1991 and in final form 23 June 1992.

REFERENCES

Alcayaga, C., X. Cecchi, O. Alvarez, and R. Latorre. 1989. Streaming potential in Ca^{2+}-activated K^+ channels from skeletal and smooth muscle. *Biophys. J.* 55:367–371.

Barry, P. H., and A. B. Hope. 1969. Electro-osmosis in Chara and Nitella cells. *Biochim. Biophys. Acta.* 193:124–128.

Bertl, A. 1989. Current-voltage relationships of a sodium-sensitive potassium channel in the tonoplast of Chara corallina. *J. Membr. Biol.* 109:9–19.

Finkelstein, A. 1987. Water Movement through Lipid Bilayers, Pores, and Plasma Membranes. Theory and Reality. John Wiley & Sons, Inc., New York 228 pp.

Gutknecht, J. 1967. Membranes of Valonia ventricosa: apparent absence of water-filled pores. *Science* (Wash. DC). 158:787–788.

Hamill, O. P., A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth. 1981. Improved patch-clamp techniques for high resolution current recording from cells and cell-free membrane patches. *Pfluegers Arch.* 391:85–100.

Hedrich, R., and J. I. Schroeder. 1989. The physiology of ion channels and electrogenic pumps in higher plants. *Annu. Rev. Plant Physiol.* 40:539–569.

Homblé, F. 1987. A tight-seal whole cell study of the voltage-dependent gating mechanism of K^+ channels of protoplasmic droplets of Chara corallina. *Plant Physiol.* 84:433–437.

Homblé, F., J. Ferrier, and J. Dainty. 1987. Voltage-dependent K^+-channel in protoplasmic droplets of Chara corallina. A single channel patch clamp study. *Plant Physiol.* 83:53–57.

Homblé, F., and B. Fuks. 1991. Quantitative analysis of single K^+ channels in the tonoplast of Chara corallina: selectivity and TEA blockade. *J. Plant Physiol.* 137:729–733.

Kamiya, N., and M. Tazawa. 1956. Studies on water permeability of a single plant cell by means of transcellular osmosis. *Protoplasma.* 46:394–422.

Kourie, J. I., and G. P. Findlay. 1990. Ionic currents across the plasmalemma of Chara inflata cells. I. Osmotic effects of sorbitol on K^+, Cl^- and leak currents. *J. Exp. Bot.* 41:141–150.

Laver, D. R., and N. A. Walker. 1987. Steady-state voltage-dependent gating and conduction kinetics of single K^+ channels in the membrane of cytoplasmic drops of Chara australis. *J. Membr. Biol.* 100:31–42.

Laver, D. R., K. A. Fairley, and N. A. Walker. 1989. Ion permeation in a K^+ channel in Chara australis: direct evidene for diffusion limitation of ion flow in a maxi-K channel. *J. Membr. Biol.* 108:153–164.

Laver, D. R., and N. A. Walker. 1991. Activation by Ca^{2+} and block by divalent ions of the K^+ channel in the membrane of cytoplasmic drop from Chara australis. *J. Membr. Biol.* 120:131–139.

Levitt, D. G., S. R. Elias, and J. M. Hautman. 1978. Number of water molecules coupled to the transport of sodium, potassium and hydrogen ions via gramicidin, nonactin or valinomycin. *Biochim. Biophys. Acta.* 512:436–451.

Levitt, D. G. 1990. Streaming potential: continuum expression applicable to very small nonuniform ion channels. *J. Chem. Phys.* 92:6953–6957.

Lühring, H. 1986. Recording of single K^+ channels in the membrane of cytoplasmic drop of Chara australis. *Protoplasma.* 133:19–28.

Miller, C. 1982. Coupling of water and ion fluxes in a K^+-selective channel of sarcoplasmic reticulum. *Biophys. J.* 38:227–230.

Rosenberg, P. A., and A. Finkelstein. 1978. Interaction of ions and water in gramicidin A channels. *J. Gen. Physiol.* 72:327–340.

Sakano, K., and M. Tazawa. 1986. Tonoplast origin of the membrane of cytoplasmic droplets prepared from Chara internodal cells. *Protoplasma.* 131:247–249.

Tyerman, S. D., and G. P. Findlay. 1989. Current-voltage curves of single Cl- channels which coexist with two types of K^+ channel in the tonoplast of Chara corallina. *J. Exp. Bot.* 40:105–117.

Wayne, R., and M. Tazawa. 1988. The actin cytoskeleton and polar water permeability in characean cells. *Protoplasma.* 2(Suppl.):116–130.

Wayne, R., and M. Tazawa. 1990. Nature of the water channels in internodal cells of Nitellopsis. *J. Membr. Biol.* 116:31–39.