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Activation by Ca^{2+} and Block by Divalent Ions of the K^+ Channel in the Membrane of Cytoplasmic Drops from *Chara australis*

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Summary. Patch-clamp studies of cytoplasmic drops from the charophyte *Chara australis* have previously revealed K^+ channels combining high conductance (170 pS) with high selectivity for K^+, which are voltage activated. The cation-selectivity sequence of the channel is shown here to be: $K^+ > Rb^+ > NH_4^+ \gg Na^+$ and Cl^-. Divalent cytosolic ions reduce the K^+ conductance of this channel and alter its K^+ gating in a voltage-dependent manner. The order of blocking potency is $Ba^{2+} > Sr^{2+} > Ca^{2+} > Mg^{2+}$. The channel is activated by micromolar cytosolic Ca^{2+}, an activation that is found to be only weakly voltage dependent. However, the concentration dependence of calcium activation is quite pronounced, having a Hill coefficient of three, equivalent to three bound Ca^{2+} needed to open the channel. The possible role of the Ca^{2+}-activated K^+ channel in the tonoplast of *Chara* is discussed.

Key Words Ca^{2+}-activated K^+ channel · patch clamp · *Chara* · ion block

Introduction

One of the best-characterized ion channels from a plant cell is the high conductance (170 pS) K^+ channel found in the membrane surrounding cytoplasmic drops from *Chara australis* (Lühring, 1986; Laver & Walker, 1987; Bertl, 1989; Laver, Fairley & Walker, 1989; Tyerman, Findlay & Terry, 1989). The cytoplasmic drop membrane is largely derived from tonoplast, according to fluorescence labeling studies (Lühring, 1986; Sakano & Tazawa, 1986; M.R. Wilson and D.R. Laver, *unpublished data*).

This *Chara* channel has strong similarities in its ion permeation and gating kinetics to the large conductance Ca^{2+}-activated K^+ channels (maxi-K or BK) in animal cells (Laver & Walker, 1987; Laver et al., 1989). These similarities led us to investigate whether this *Chara* channel is Ca^{2+} activated and to compare the ion selectivity and Ca^{2+}-dependent kinetics of this channel with those of other K^+ channels in plants and animals.

In animal cells maxi-K channels play roles in excretion and excitability (Blatz & Magleby, 1987). In some neurons (Adams et al., 1982) and chromaffin cells (Marty, 1983), for example, they are believed to open in response to Ca^{2+} which flows into the cytoplasm through voltage-dependent Ca^{2+} channels when the membrane PD is depolarized during an action potential. The K^+ flux through these channels aids in repolarizing the membrane PD, thus closing the Ca^{2+} channels. The fact that the *Chara* tonoplast exhibits excitability (Findlay & Hope, 1964) suggests a similar role for the Ca^{2+}-activated K^+ channel, that of regulating cytosolic $[Ca^{2+}]$ during an action potential. The time course of the tonoplast action potential is affected by divalent cations such as Ba^{2+}, Sr^{2+} and Mg^{2+} (Kikuyama, 1986). Therefore we examine the effects of these cations to elucidate the role of this K^+ channel in the *Chara* tonoplast.

Materials and Methods

Cytoplasmic drops were formed by the effusion of cytoplasm from internodal cells of *C. australis* R. Br. as previously described (Kamiya & Kuroda, 1957). Measurements were carried out at 23 ± 2°C. The patch-clamp apparatus and fabrication of patch-clamp pipettes is described elsewhere (Laver & Walker, 1987). Measurements of single-channel currents were made using a patch-clamp amplifier (List EPC-7). Current and voltage signals were recorded on video tape (Sony SL-HF150) using pulse code modulation (Sony PCM-501 ES).

Inside-out patches were produced by sealing fire-polished pipettes against the drop membrane. The patches could be excised by moving the pipette tip away from the drop. Sometimes the membrane would form a vesicle in the pipette tip which could be transformed into an inside-out patch by briefly exposing the pipette tip to air. It is presumed that the side of the channel facing the cytoplasm of the intact cell also faces the cytoplasmic interior of the drop.

The membrane potential difference (membrane PD) is defined with respect to the outside (vacuolar side) of the membrane as zero. Positive current indicates positive charge flowing from the inside (cytoplasmic side) to the outside of the membrane. Reversal potentials were obtained by low-order polynomial interpolation of the current-voltage data. Net electrode-solution potentials and solution-junction potentials are accounted for when deriving the membrane potentials (Laver et al., 1989).

The raw current data was filtered at 5 kHz (4 pole, Bessel) and analyzed for event durations using the software package IPROC2 (from Dr. C. Lingle, Florida State University). Frequency distributions of event durations were generated from single-channel records as described by Laver and Walker (1987).

During measurements of the Ca^{2+}-dependent gating kinetics of the channel in inside-out patches, the bathing medium in which cytoplasmic drops were produced and the pipette filling solution both consisted of 150 mM KCl plus 0.5 mM $CaCl_2$. The bath was perfused with Ca^{2+}-buffered solutions containing 150 mM KCl, 2 mM EGTA (pH 6.9) or 10 mM EGTA (pH 7.3), 5 mM TES.The *p*Ca was adjusted with $CaCl_2$ and the pH with KOH. The free-Ca^{2+} concentration was calculated using the algorithm of the program COMICS (Perrin & Sayce, 1967) with reaction coefficients from Martell and Smith (1974). Solution exchange in the bath was achieved using two syringe barrels mounted so that bath perfusion and waste withdrawal could be maintained at equal rates. In this way sufficient exchange of the bath solution (volume 4 ml) was achieved with 20 ml of perfusion medium.

Divalent cytosolic ion blocking experiments were made on channels in excised inside-out membrane patches in symmetric 150 mM KCl. The bath solution also usually contained 0.1 mM $CaCl_2$ and 5 mM of the divalent ion in the form of a chloride salt.

Results

Ca^{2+}-Activation Kinetics

The effect of the cytosolic Ca^{2+} concentration ($[Ca^{2+}]$) on the channel activity was measured using excised inside-out membrane patches in a bath containing Ca^{2+}-buffered solutions (*see* Materials and Methods). Reducing $[Ca^{2+}]$ to less than 0.1 μM reversibly abolishes most of the channel activity (Fig. 1). The open probability of the channels, relative to that of channels in the same patch at 0.5 mM Ca^{2+}, shown in Fig. 2, is seen to be $[Ca^{2+}]$ dependent. Thus the mean probability of channel being open is strongly dependent on the cytosolic $[Ca^{2+}]$ over the range 0.1 to 10 μM. This data was obtained from 20 patches each containing up to 5 individual channels. The relative open probabilities were calculated from the mean current through the channels in the patch over several minutes allowing for unitary current differences. The Ca^{2+}-binding affinity was determined from the concentration of Ca^{2+} required to achieve 50% activation of the channel. It varies from 0.5 to 5 μM between patches at -50 mV with a mean value which is voltage dependent, decreasing from 1.5 μM at -100 mV to 0.3 μM at $+50$ mV (Fig. 2). Figure 3 shows the $[Ca^{2+}]$-dependent channel activity obtained from a single, long-lived inside-out membrane patch over a wide range of $[Ca^{2+}]$. The $[Ca^{2+}]$ dependence in Fig. 3 appears stronger than in Fig. 2 because averaging data from several patches tended to smear out the concentration dependence along the concentration axis.

Single-channel recordings which could be used to analyze the Ca^{2+}-dependent gating kinetics were obtained from five inside-out patches. Figure 4 shows the frequency distributions of closed times for a channel at $[Ca^{2+}]$ of 1 mM and 0.1 μM. At the lower $[Ca^{2+}]$ the record shows relatively long closed periods with a mean duration, in the case of Fig. 4, of about 100 msec. This is accompanied by a slightly lower mean open time of the channel.

Monovalent Cation Selectivity

Figure 5 shows the current-voltage (*I/V*) characteristic for this channel in excised patches (inside-out and outside-out) with various cations in the bath and pipette. The conductance of the *Chara* K^+ channel from the data in Fig. 5 is 170 pS.The ion-selectivity sequence and ion permeabilities relative to K^+, deduced from the current reversal potentials in Fig. 5, are: K^+ (1) > Rb^+ (0.33 ± 0.03) > NH_4^+ (0.11 ± 0.05) ≫ Na^+ (0.02 ± 0.04). In the derivation of these relative cation permeabilities we allowed for a relative Cl^- permeability of 0.015 ± 0.01 (obtained from Laver et al., 1989).

Blockade by Divalent Cations

In inside-out patches the divalent ions Mg^{2+}, Ca^{2+}, Sr^{2+} and Ba^{2+} in the cytosolic solution (the bath) interfere with K^+ permeation through the channel in two ways. First, they cause a voltage-dependent decrease in the unitary K^+ current (Fig. 6) and, second, they decrease the frequency of channel opening (Fig. 7*a* and *b*). Both forms of block are most pronounced at positive membrane PD, and their potency increases with increasing atomic weight of the blocking ion. The blocking effect of Ba^{2+} is the same whether $[Ca^{2+}]$ is 1 or 100 μM (Fig. 7*b*).

Figure 8*a* and *c* shows the effect of 5 mM cytosolic Ba^{2+} on the channel activity when the excised patch is hyperpolarized from a holding potential of 100 to -100 mV (Fig. 8*a*) and also when depolarized from a holding potential of -50 to 50 mV (Fig. 8*c*). The presence of Ba^{2+} and Sr^{2+} during depolarization

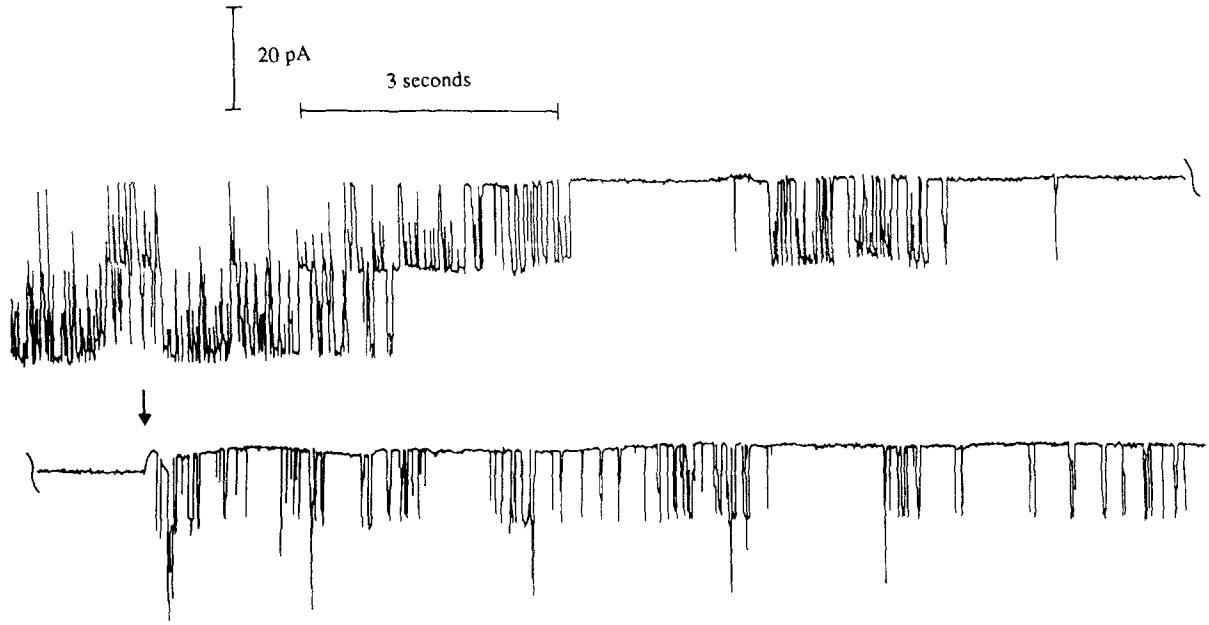

Fig. 1. A single-channel recording (membrane PD = −100 mV) showing the effect of perfusing the bath, originally containing 0.5 mM Ca^{2+}, with a low-$[Ca^{2+}]$ (<1 nM) buffer at time zero. The break in the record contains several minutes of recording with no channel activity. After this time the activity was re-established when a bolus of Ca^{2+} was added to the bath (marked by the arrow)

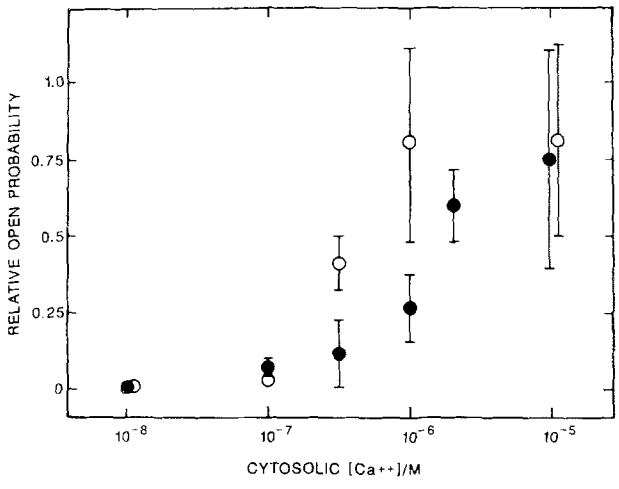

Fig. 2. Ca^{2+}-activation characteristics of the large-conductance K^+ channel in *Chara* derived from 20 patches. The Ca^{2+} dependence of the open probability of the channel at membrane potentials 50 mV (○) and −100 mV (●) relative to that at $[Ca^{2+}] = 0.5$ mM. The relative open probabilities were derived by taking the ratio of the mean current through all the K^+ channels in each patch before and after perfusion of the bath with a low $[Ca^{2+}]$ buffer. Ca^{2+} at these concentrations does not significantly block the K^+ channel

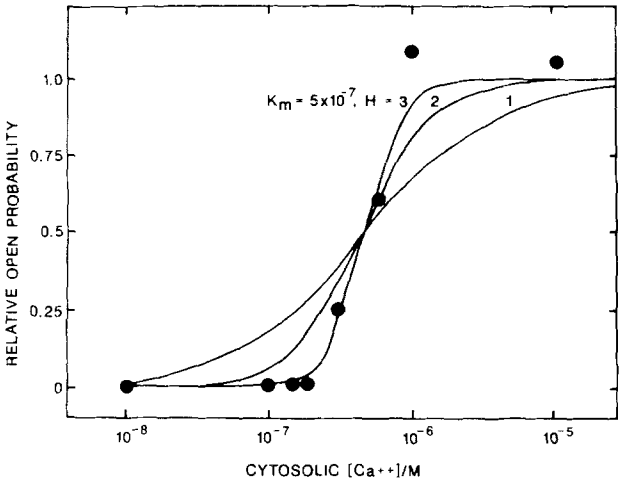

Fig. 3. The Ca^{2+} dependence of the open probability of one channel in a long-lived patch at a membrane PD of −50 mV. The solid curves are predictions of the relative open probability of the channel, P, using the following equation with values of the Hill coefficient $H = 1$, 2 and 3:

$$P = (1 + (K_{mCa}/[Ca^{2+}])^H)^{-1}$$

switches the channel off. However, the activity of the channel was often observed to be transiently promoted upon repolarization (*see* Fig. 8*a* and *b*). The data in Fig. 8*c* show that the reduction of the channel activity at positive membrane PD is due to

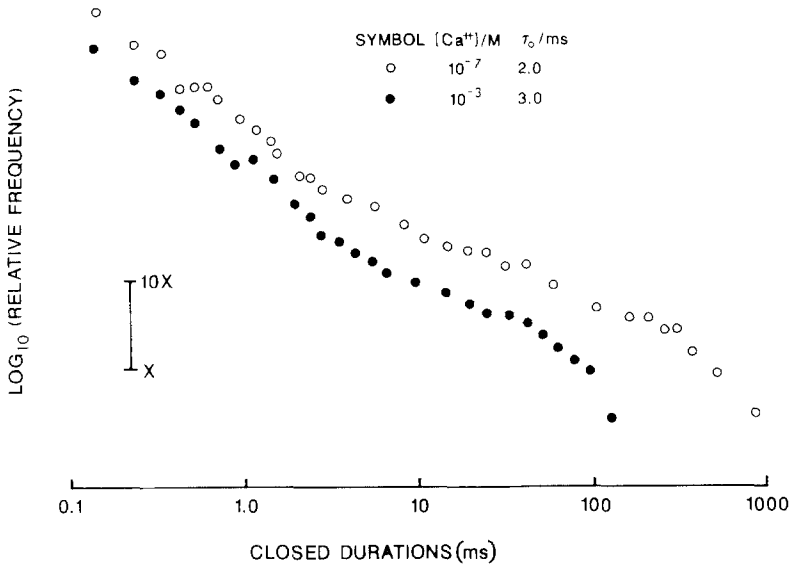

Fig. 4. The closed duration frequency distribution of a *Chara* channel at high and low $[Ca^{2+}]$ with a membrane PD of −100 mV. The data has been translated vertically for clarity and so the vertical axis represents a relative frequency. Note the increased frequency of long closed periods ($t > 100$ msec) and the slight reduction in the mean open duration, τ_o, at lower $[Ca^{2+}]$

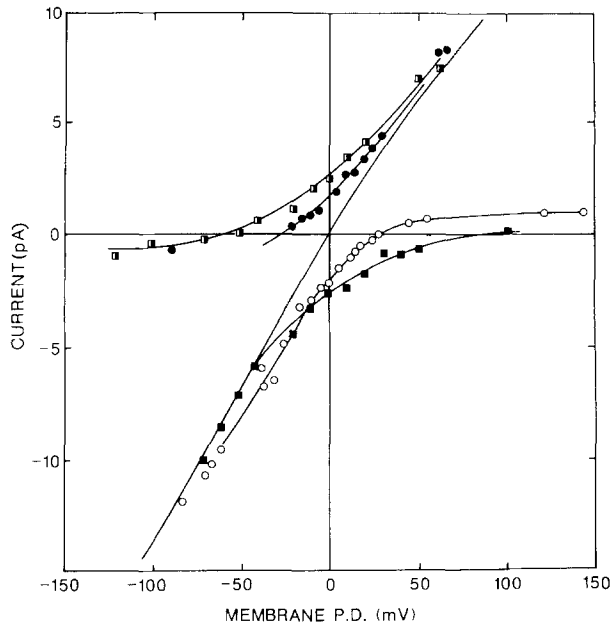

Fig. 5. Current-voltage characteristics for the K^+ channel in excised patches from cytoplasmic drops in the presence of different monovalent cations. The solid curves are low-order polynomials fitted to the data using least squares. The ionic concentrations in the bathing solutions (cytosolic, outside; in mM) and reversal potentials associated with each symbol are: ●—150 NaCl, 150 KCl, 84 ± 20 mV; ○—150 RbCl + 1 $CaCl_2$, 150 KCl + 0.1 $CaCl_2$, 27 ± 3 mV; ●—150 KCl + 1 $CaCl_2$, 150 RbCl + 1 $CaCl_2$, −25 ± 3 mV; ○–150 NH_4Cl + 0.5 $CaCl_2$, 150 KCl, −52 ± 8 mV; (*data points not shown*) symmetric 150 KCl, 0 ± 3 mV. Where the value of $[Ca^{2+}]$ is not stated it is less than 10 μM. Positive current is defined here as positive charge moving from the cytoplasmic side of the patch to the outside, and membrane potentials are with respect to the outside

long ion-induced closures of the channel. The time course of the onset of the slow block at positive potentials is resolvable by the recording apparatus though relief of the block during hyperpolarization of the membrane is quite rapid.

Discussion

Monovalent Cation Permeability

In a recent review of plant ion channels Tester (1990) found that, generally speaking, K^+ channels in the tonoplast have a higher conductance and lower selectivity than those in the plasmalemma. Tonoplast K^+ channels with high conductance have been found in *Chara buckellii* (Bisson, Tyerman & Findlay, 1989), barley cells (Kolb, Köhler & Martinoia, 1987), sugarbeet (Coyaud et al., 1987) and in a variety of other plant cells (*see* review by Hedrich et al., 1988). The selectivity for K^+ over Na^+ and Cl^- for these channels is low ($P_{Na}/P_K = 0.17$ in barley; Kolb et al., 1987: $P_{Na}/P_K = 1$, $P_{Cl}/P_K = 0.17$ in sugarbeet; Hedrich & Neher, 1987). The conductance of most plasmalemma K^+ channels is on the other hand less than 70 pS (in 100 mM KCl), while such channels have high selectivity (e.g., *C. australis;* Tester, 1988; and in *Vicia faba* guard cells; Schroeder, 1988).

The tonoplast K^+ channel in *C. australis* is unusual in that it has both high K^+ selectivity ($P_{Na}/P_K = 0.02$) and K^+ conductance (170 pS). A channel with these properties has recently been found in cytoplasmic drops from the characeae *Lamprothamnium succinctum* (Katsuhara, Mimura & Tazawa, 1989). Another plant channel that may also reveal

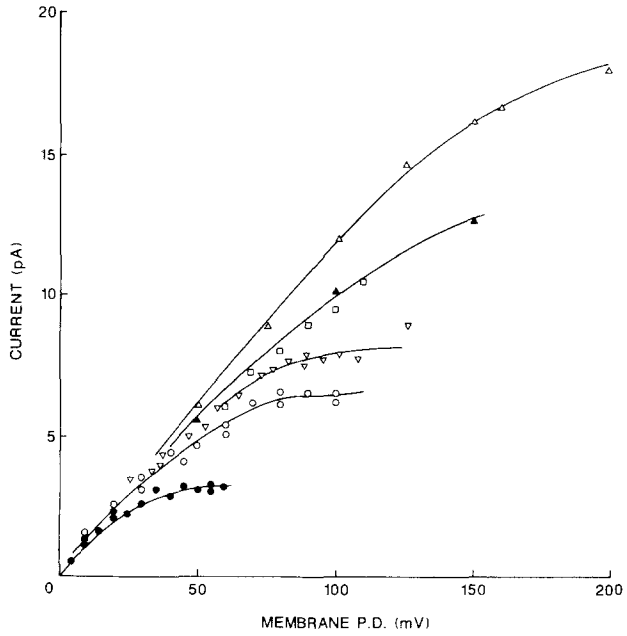

Fig. 6. The unitary K^- current in excised inside-out patches with 0.1 mM $CaCl_2$ in the pipette and in symmetric 150 mM KCl. The divalent ion concentrations in the bath (cytosolic side of the patch) associated with each symbol are: △—1 μM Ca^{2+} (Ca^{2+}-buffered solution containing 2 mM EGTA, *see* Materials and Methods); ▲—0.1 mM Ca^{2+}, ▽—5 mM Ca^{2-}, □—5 mM Mg^{2+} + 0.1 mM Ca^{2+}; ○—5 mM Sr^{2+} + 0.1 mM Ca^{2+}; ●—5 mM Ba^{2-} + 0.1 mM Ca^{2+}

similar ion permeation properties on further investigation is the K^+ channel in the plasmalemma of *Acetabularia* (120 pS in 130 mM KCl + 5 mM $MgCl_2$ + 5 mM $CaCl_2$; Bertl & Gradmann, 1987). Considering the blocking effect of Ca^{2+} and Mg^{2+} on the *Chara* channel it is likely that the K^+ channel in *Acetabularia* in the absence of these ions would have a larger conductance than 120 pS, more like that of the *Chara* channel. The relative Na^+ permeability of the *Acetabularia* plasmalemma indicates that the 120 pS K^+ channel also has the same relative Na^+ permeability as that found for the *Chara* channel.

In combining high K^+ conductance and high K^+ selectivity the *Chara* channel more closely resembles the animal maxi-K channel (*see* reviews by Latorre & Miller, 1983; Latorre et al., 1989) than the other K^+ channels so far characterized in noncharophyte plants. For example, maxi-K channels in rat myotubes and rabbit T-tubules have the following selectivity sequence and relative permeabilities (Blatz & Magleby, 1984): Tl^+ (1.2) > K^+ (1) > Rb^+ (0.67) > NH_4^+ (0.11) ≫ Na^+ (<0.01), Li^+ (<0.01), Cs^+ (<0.05). The main difference between *Chara* and animal maxi-K channels is that *Chara* channels are more selective for K^+ over Rb^+ (their relative Rb^+ permeabilities are 0.33 and 0.67).

Dependence on Ca^{2+} Concentration

The observed variation in the Ca^{2+} sensitivity of this *Chara* channel from channel to channel is similar to that observed for other Ca^{2+}-activated K^+ channels (Moczydlowski & Latorre, 1983). The cause of the variation is not clear. It may result from variations in the local [Ca^{2+}] arising from the competing effects diffusion of Ca^{2+} through the membrane from the patch pipette and the chelating of Ca^{2+} by EGTA in the bath.

Models describing the Ca^{2+}-dependent gating kinetics of K^+ channels (e.g., Moczydlowski & Latorre, 1983) involve a gate that opens when Ca^{2+} binds to the channel protein. These models explain the observed bursts of channel openings and gaps between these bursts as times when sufficient Ca^{2+} is or is not bound. Figure 4 shows that the frequency distribution of closed durations is affected by the cytosolic [Ca^{2+}] only at relatively long durations. These long [Ca^{2+}]-dependent closures represent times when the Ca^{2+} gate is shut. Because it is not possible to identify which individual channel closures are due to the Ca^{2+} gate they are defined here as shut intervals exceeding a threshold duration. The threshold value is determined using the criterion of Magleby and Pallotta (1983*a*) which is based on the cross over of exponential components within the closed duration frequency distribution. Mean burst durations so defined (i.e., the mean lifetime of bound Ca^{2+}) with [Ca^{2+}] in the range of 0.1 to 10 μM varied from 20 to 100 msec between different patches.

Ca^{2+}-activation of the channel has a strong concentration dependence: the Hill coefficient of 3 (Fig. 3) indicates that the binding of three Ca^{2+} ions are involved in the operation of the Ca^{2+} gate.

The Woodhull (1973) model of ion binding predicts the following exponential voltage dependence for the ion binding affinity, K_m:

$$K_m = K_m^o \exp(\delta z e V/kT) \quad (1)$$

where V is the membrane PD, K_m^o is the equilibrium binding affinity, z is the valency of the binding ion, e and is the electronic charge and δ is the fractional electrical distance of the binding site through the pore. Provided the electric field is constant along the pore δ is equal to the fractional physical distance of the binding site along the pore.

The voltage-dependent Ca^{2+}-activation kinetics is consistent with the following relationship:

$$K_{mCa} = 5 \times 10^{-7} \exp(0.25\, eV/kT) \quad (2)$$

where δ for the Ca^{2+} gate, $\delta_{Ca} = 0.13$. However, if three Ca^{2+} bind to the protein then the mean δ for these sites is one-third of δ_{Ca} (approximately 0.04).

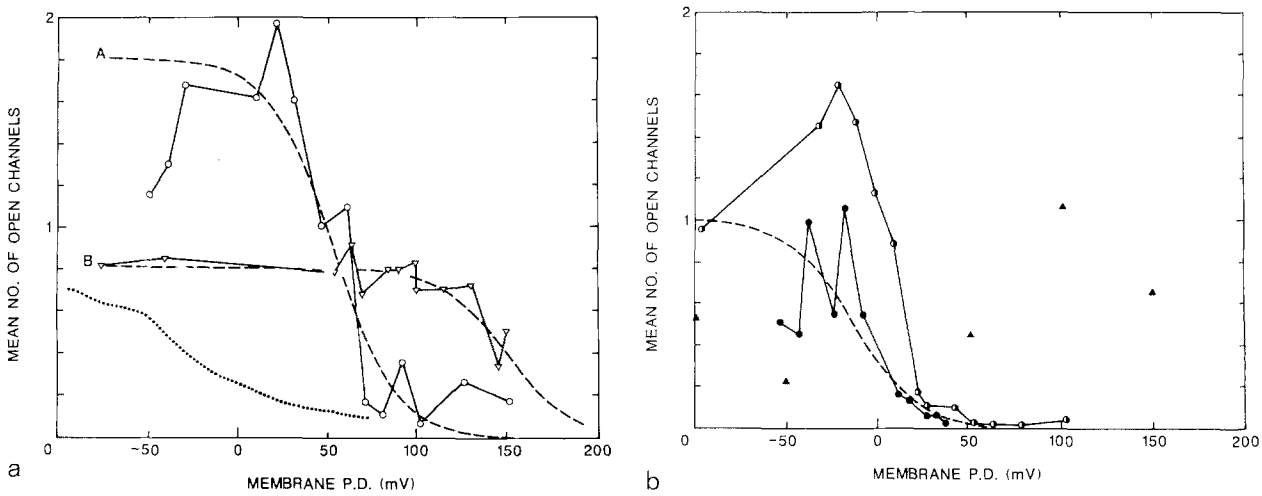

Fig. 7. The mean number of open channels in excised inside-out patches over a range of membrane PD. The mean number of open channels is calculated by dividing the average K^+ channel current through the patch over 10 sec by the unitary current. The patches were in symmetric 150 mM KCl + 0.1 mM $CaCl_2$. The concentrations of other divalent ion species in the bath associated with each symbol are: (*a*) ▽—5 mM Ca^{2+}; ○—5 mM Sr^{2+}; (*b*) ○—5 mM Ba^{2+} + 1 μM Ca^{2+}; ●—5 mM Ba^{2+} + 100 μM Ca^{2+}; ▲—100 μM Ca^{2+}. The data shown here were from patches containing two to four channels except that showing the Ca^{2+} block where only one channel was present. Mg^{2+} did not significantly reduce the open-channel activity over the experimental voltage range. The dashed lines are the predicted values of the mean number of open channels in a patch, P_o, based on the following equation:

$$P_o = N\,(1 + [X^{2+}]/K_{mX})^{-1}$$

where K_m (a function of the parameters δ and K^o_{mX}) is the voltage-dependent affinity of the blocking ion X^{2+} given by Eq. (1) and N is a normalizing constant which in part depends on the number of channels in the patch. The denominator in this equation is related to the fractional occupancy of X^{2+} in the pore. The parameter values are: (*a*) line *A*: $N = 1.8$, $\delta = 0.7$, $K^o_{mSr} = 100$ mM; line *B*: $N = 0.8$, $\delta = 0.7$, $K^o_{mCa} = 25$ M; (*b*) $N = 1$, $\delta = 0.7$, $K^o_{mBa} = 2.5$ mM. The dotted line shows the mean open probability of approximately 20 individual K^+ channels in a drop-attached patch derived from the data in Figs. 2 and 3 from Laver and Walker (1987). This data shows that the cytoplasm affects control over activity of the K^+ channel

Thus it appears that the Ca^{2+} binding sites responsible for gating the channel are superficially located on the cytosolic side of the channel.

Ca^{2+}-activated K^+ channels with high K^- conductance (100 pS in 100 mM KCl) have been found in the tonoplast membrane of the characeae *L. succinctum* (Katsuhara et al., 1989). Though the Ca^{2+}-dependent properties of this channel have not been characterized it is likely that it will have similar kinetics to the *Chara* channel. Other Ca^{2+}-activated K^+ channels with conductances of 60 to 80 pS (in 100 mM KCl) have also been detected in sugarbeet vacuoles (Hedrich & Neher, 1987). The *Chara* and the sugarbeet channels both have Ca^{2+} binding affinities of about 1 μM. However, the Hill coefficient of Ca^{2+} binding to the sugarbeet K^+ channel is approximately 1 and so is significantly lower than that found for the *Chara* K^+ channel. A K^+ channel with a conductance of 35 pS (in 100 mM KCl) has been found in endosperm plasma membrane from *Haemanthus* and *Clivia* which open with 200 μM internal Ca^{2+} (Stoeckel & Takeda, 1989).

The Hill coefficient for the *Chara* channel is typical of those found for maxi-K channels which found to be either 2 or 3 (Barrett, Magleby & Pallotta, 1982; Magleby & Pallotta, 1983*b*; Moczydlowski & Latorre, 1983; Oberhauser, Alvarez & Latorre, 1988). The Ca^{2+} binding affinity of maxi-K channels in different animal cell types vary considerably, ranging from 1 nM to 1 μM (Latorre et al., 1989). The Ca^{2+} binding affinity of the K^+ channel in *Chara* is at the low end of the range at 1 μM. However, the voltage dependence of Ca^{2+} binding to the *Chara* channel is relatively weak compared to that normally found in maxi-K channels; a 10-fold change in Ca^{2+} binding being effected by a voltage change of about 200 mV (*cf.* a range of 40–110 mV for maxi-K channels in animals; Latorre et al., 1989). This difference implies that the Ca^{2+} binding sites in the *Chara* channel are located nearer to the cytosol than those in animal maxi-K channels.

Block by Divalent Cations

The results shown in Fig. 6 indicate that divalent ions can block the K^+ channel by two kinetically distinct mechanisms: one which gives rise to an at-

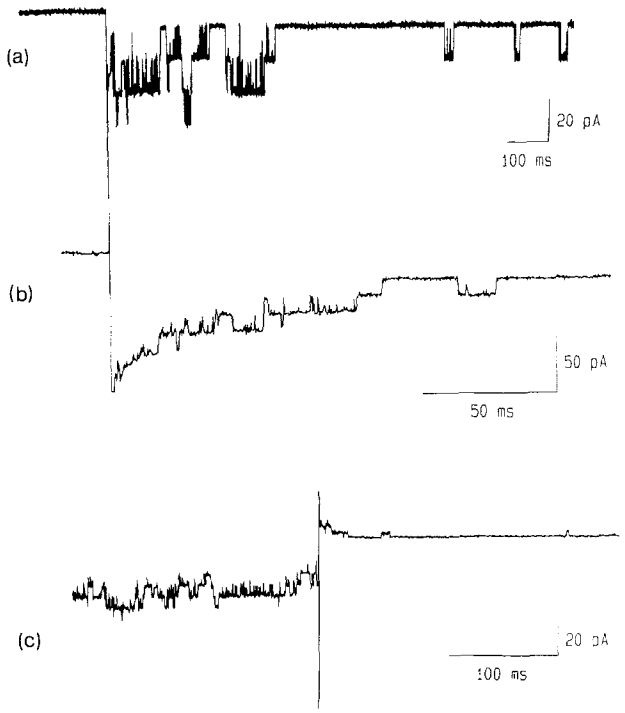

Fig. 8. The time course of unitary currents in an excised inside-out patch in symmetric 150 mM KCl upon which in imposed a step change in membrane PD. (*a*) The bath contains 5 mM $BaCl_2$ and the membrane PD is changed from 100 to −100 mV. Prior to the repolarization the channel is blocked by Ba^{2+} whereas at negative membrane PD Ba^{2+} does not significantly block the channel. After the step change the open-channel activity is greater in the first few hundred milliseconds than that found in steady state. (*b*) $SrCl_2$ (5 mM) is on the cytosolic side of the patch, and the membrane PD is changed from 100 to −100 mV. Sr^{2+} and Ba^{2+} have a similar transient effect on channel open activity upon step repolarization. (*c*) $BaCl_2$ (5 mM) is on the cytosolic side of the patch and the membrane PD is changed from −50 to 50 mV. The Ba^{2+}-induced reduction in channel conductance is immediate; however, its effect on channel gating takes several tens of milliseconds

tenuation of the unitary current and another which produces long closures of the channel. The reduction in the current is presumably due to a rapid block that is not resolved by the recording apparatus. The voltage-dependent nature of the ion block is consistent with a mechanism whereby blocking ions must overcome an energy barrier which is located within the electric field in the pore (Woodhull, 1973). Analysis of the internal Ca^{2+} blockade of the *Chara* channel (Laver, 1990) indicates that divalent cytosolic ion block is governed by multi-ion kinetics (i.e., that more than one ion can simultaneously occupy the pore).

The reduction in channel open activity induced by divalent ions (Fig. 7*a* and *b*) could be due to either (*i*) divalent ions binding to sites within the pore and so directly competing with K^+ for the permeation pathway or (*ii*) competition between Ca^{2+} and other divalent ions for the sites which activate the Ca^{2+} gate and so inactivating the channel. However, no competition between Ba^{2+} and Ca^{2+} could be discerned from the data in Fig. 7*b* which shows that varying $[Ca^{2+}]/K_{mCa}$ from 1 to 100 has no effect on the Ba^{2+} block. Therefore it appears that Ba^{2+} block is due to Ba^{2+} competing with K^+ for the pore. The voltage-dependence of the block is quite pronounced and its onset is shifted to more negative membrane PD as the atomic weight of the blocking ion increases.

Similar fast and slow Ca^{2+} blocking kinetics of the maxi-K channel from rabbit T-tubule has also been reported by Latorre (1986). For the slow block values of δ for Ba^{2+}, Sr^{2+} and Ca^{2+} are all approximately 0.7 which is similar to that of the slow blocking kinetics of Ca^{2+} ($\delta = 0.65$) and Ba^{2+} ($\delta = 0.8$) observed in the maxi-K channel from rabbit muscle (Vergara & Latorre, 1983). However, the zero-voltage binding affinities for the *Chara* channel ($K^o_{mBa} = 2.5$ mM, $K^o_{mSr} = 100$ mM and $K^o_{mCa} = 25$ M) are 100-fold larger than that found in rabbit muscle ($K^o_{mBa} = 36$ μM and $K^o_{mCa} = 290$ mM; Vergara & Latorre, 1983).

At low divalent concentrations the open-channel activity is reasonably constant over the voltage range of ±150 mV. This differs from that measured in drop-attached patches (*cf.* Fig. 7*a* and *b*) where the open-channel activity decreased at positive membrane PD. The maximum likely concentration of Mg^{2+} in the drops will not be greater than found in the intact cell ($[Mg^{2+}] < 5$ mM; Okihara & Kiyosawa, 1988) and that for Ca^{2+} will not exceed that found in the bath ($[Ca^{2+}] < 5$ mM). At these concentrations these ions would not sufficiently alter the channels gating or permeation kinetics to account for the difference between excised and attached patches. Thus it appears that other constituents of the cytoplasm affect control over the gating of the channel at depolarized membrane PD.

Role of the Ca^{2+}-Activated K^+ Channel in the *Chara* Tonoplast

The tonoplast of the charophyte internode is involved in the action potential; Findlay and Hope (1964) showed that the tonoplast PD ($V_{cytoplasm} - V_{vacuole}$) changes from about −15 mV at rest to about −60 mV when excited, recovery taking several seconds. The approximate ion equilibrium PDs (eg., V_K for K^+) for the major ions are: K^+, −15 mV; Na^+, +60 mV; Ca^{2+}, +300 mV; Cl^-, −70 mV. Thus the tonoplast action potential, like the plasmalemma one, goes from near V_K to near V_{Cl} and back.

The tonoplast action potential is not electrically triggered (Findlay, 1970; Kikuyama & Tazawa, 1976), but is chemically initiated by the plasmalemma action potential. This produces a rise in cytosol $[Ca^{2+}]$ which activates tonoplast Cl^- channels (Kikuyama, 1986). The return to a PD near V_K may possibly involve opening of K^+ channels, since the closing of the Cl^- channels does not appear to be due to a voltage-dependent inactivating mechanism. Since the tonoplast action potential can be inverted by removing vacuolar chloride (Kikuyama & Tazawa, 1976; Shimmen & Nishikawa, 1988).

Externally applied Ba^{2+} (5 mM) prolongs the tonoplast action potential during the initial cell excitation and then abolishes tonoplast excitability (Kikuyama, 1986). Although cytosolic Ba^{2+} is the most potent K^+ channel blocker of the divalent ions tested it had no significant blocking effect on the Ca^{2+}-activated K^+ channel of the tonoplast at a concentration of 5 mM and at physiological membrane PDs. The effect of Ba^{2+} on the action potential must be by a mechanism other than a direct block of this tonoplast K^+ channel.

If the K^+ channels are open only during the action potential, as their activation characteristics suggest it is possible that they might help restore the PD to V_K at the end of the action potential, since we have shown that in vivo, or to be exact *in droppo*, the channels are open near -60 mV and mostly closed near 0 mV (Fig. 7*a*). However in order to account for the long delay before the action potential ends the K^+ channels would need to be opened by higher cytosolic $[Ca^{2+}]$ than the Cl^- channels. Alternatively, they may regulate the tonoplast PD during excitation by controlling the relative K^+ and Cl^- permeability of the membrane.

Although some authors ascribe no explicit function to the tonoplast action potential, it is possible to see a function in providing a flow of Ca^{2+} from the vacuole to the cytosol, through voltage-activated Ca^{2+} channels, or in providing a flow of KCl into the cytoplasm to replace that lost to the outside during plasmalemma excitation. If one of these conjectures is correct, the Ca^{2+}-activated K^+ channels could regulate cytosol $[Ca^{2+}]$ or cytosol $[Cl^-]$. They could not work by clamping the tonoplast PD at V_K, so preventing excitation, since the tonoplast is Ca^{2+} excited, but they could regulate the membrane PD during excitation and so regulate the flux of Ca^{2+} and/or of Cl^-.

This suggested role is different from, but not incompatible with, the role ascribed to similar K^+ channels in the tonoplast of *Lamprothamnium* by Katsuhara et al. (1989). These authors suggest that their K^+ channel is opened, simultaneously with Cl^- channels, by a rise in cytosol $[Ca^{2+}]$ which signals hyposmotic conditions, and which produces net efflux of KCl from the vacuole as well as from the cytoplasm. Such a role is in line with the known properties of the *Chara* K^+ channel, including the voltage activation. *Chara* does not osmoregulate but it does regulate its vacuolar salt concentration (Kiyosawa & Okihara, 1988), and an analogous role for K^+ channels may exist in this process, which would be compatible with the suggestions already made.

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