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Probing of pore in the *Chara gymnophylla* K^+ channel by blocking cations and by streaming potential measurements

Igor I. Pottosin

Laboratory of Membrane Biophysics, Institute of Cell Biophysics, USSR Academy of Sciences, Pushchino, 142292, USSR

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The current–voltage (I–V) relationship of single K^+ channels present in the *Chara gymnophylla* droplet membrane was studied. The channel presumably contains large mouths at both pore ends which are sufficiently wide to accomodate TEA^+ as evidenced by internal and external TEA^+ blockade. The voltage dependence of blockade by external Cs^+ and Na^+ reveals the multi-ion occupancy of the channel. The value of streaming potential (4.0 mV/osmol) measured in the *Chara* K^+ channel indicates that the channel contains up to nine water molecules in the narrow region. It is concluded that the length of this region is around 28 Å.

K^+ channel; Ca^{2+}-dependent; *Chara*; Patch-clamp; Ion block; Streaming potential

1. INTRODUCTION

Large-conductance K^+-selective channels have been previously characterized in the membrane surrounding cytoplasmic drops obtained from various *Charophytes* [1–5]. These channels could be activated by voltage and micromolar cytosolic Ca^{2+} [2–5], which is reminiscent of the properties of the large-conductance Ca^{2+}-activated K^+ channels (maxi-K_{Ca}), widespread in animal cells. K^+ channel blockers and streaming potential measurements are often used to classify channel types and to probe the channel architecture [6–9]. In the present study this approach was used to provide further insight into the structural similarities between the *Chara* K^+ channels and K^+ channels of other origins.

2. MATERIALS AND METHODS

Cytoplasmic droplets were obtained from internodal cells of *C. gymnophilla* by the method of Bertl [10] and the standard patch-clamp technique was used for detection of single K^+ channels in the isolated outside-out patches. Currents stored on magnetic tape (TEAC MRC-10c) were digitized using a Nicolett-527 signal averager and copied by an x-y plotter. Current amplitudes were evaluated by hand. All solutions contained (in mM), 100 KCl, 1.5 $CaCl_2$, 0.5 EDTA, 5 HEPES, and were titrated to pH 7.2 with KOH. Blockers to be tested were added to the bath or into the pipette filling solution. For the measurements of streaming potential, V_s, the open channel I–V relationships in the control (symmetrical 100 mM KCl) and in the presence of an osmotic gradient established by the addition of high concentrations of either urea, glycerol or sucrose in the bath, were taken and reversal potentials were estimated. The shift of reversal potential (ΔV_r) caused by non-electrolyte addition was further corrected to the change to K^+ activity (ΔV_a) as measured by a K^+-selective glass electrode. Streaming potentials were calculated as $V_s = \Delta V_r - \Delta V_a$ and plotted against solution osmolality measured by a freeze-point osmometer (Knauer).

Abbreviations: TEA^+, tetraethylammonium; TMA^+, tetramethylammonium; TBA^+, tetrabuthylammonium HEPES, 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid; EDTA, ethylenediaminetetraacetic acid.

Correspondence address: I.I. Pottosin, Laboratory of Membrane Biophysics, Institute of Cell Biophysics, USSR Acad. Sci., Pushchino (Moscow region), 142292, USSR.

3. RESULTS

An example of voltage-dependent channel's blockage by Cs^+ is shown in Fig. 1. The reduction in single-channel current by Cs^+ is enhanced by negative voltage and the open-channel currents fluctuate. This observation is consistent with the concept that the blocker temporarily occludes the pore by entering it to some distance down the electric field. This kind of interaction may produce the negative slope regions in I–V relationship of the open channel as shown in Fig. 2A for Na^+ blockade. Similar changes in the shape of I–V relationship induced by Na^+ have been previously reported for the *C. corallina* K^+ channel [10].

For the description of block voltage dependence, the data were fitted to the equation obtained from a single site model of ion blockade [11]:

$$I_{K+B}/I_K = \{1 + [B]\exp(\delta FV/RT)/k_d(0)\}^{-1} \quad (1)$$

where $I_{K+B}(V)$ and $I_K(V)$ are currents through the blocked and unblocked channel, respectively, [B] is blocker concentration, δ is a fraction of potential drop across the membrane left by the blocker in moving into the binding site, $k_d(0)$ is the apparent dissociation constant at 0 mV, and V, F, R, T have their usual meanings.

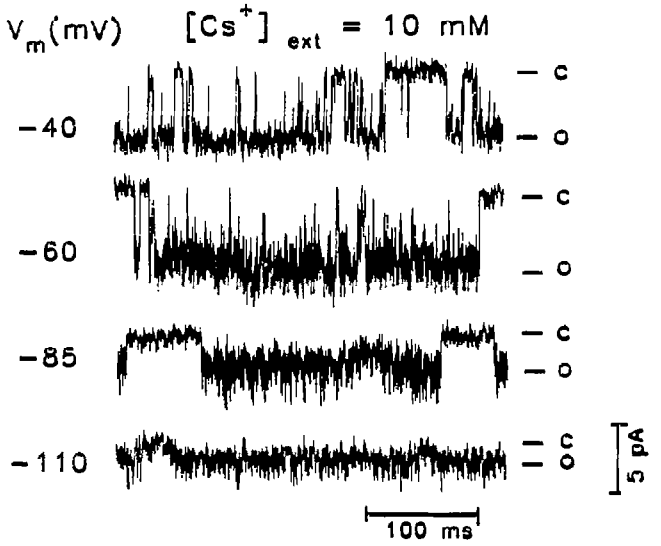

Fig. 1. Fluctuating block of a single K^+ channel in an outside-out patch by external Cs^+ (10 mM). [KCl] = 100 mM at both membrane sides (V_r=0 mV). Closed (c) and open (o) states of the channel are indicated by each record. Signals are filtered at 1 kHz.

The curves obtained at different external or internal concentrations of Na^+, Cs^+ and TEA^+ are shown in Figs. 2B and 3. The values of δ and $k_d(0)$ are given in Table I. Externally applied TMA^+ causes a lower affinity voltage-dependent block (δ= 0.2; $k_d(0)$= 125 mM);

Table I

Voltage-dependent blockade in the *C. gymnophylla* K^+ channel

Blocker	External application		Internal application	
	$k_d(0)$, mM	δ	$k_d(0)$, mM	δ
Na^+	680–5,990*	0.60–1.00*	820	0.96
Cs^+	570	1.45	150	0.20
TEA^+	30	0.02	20	0.33

* The increase of external Na^+ concentration in the range of 10–100 mM is followed by the apparent increase of the zero voltage dissociation constant, $k_d(0)$, and of electrical distance, δ. In other cases, the variations in $k_d(0)$ and δ values are insignificant (less than two-fold and by 10%, respectively) and the average values are given.

external TBA^+ was ineffective up to 100 mM concentration (not shown). The above single site model, however, could not account for some results present in Table I, namely (i) $\delta \geq 1$ found in the cases of blockade by Na^+ and by external Cs^+; and (ii) voltage dependence that varies with Na^+ concentration. Thus, one is forced to conclude that the channel is able to accept at least two ions simultaneously.

The multi-ion channels are thought to have long tunnels where ions and water are constrained to move in single file. Due to the coupling of water and ion fluxes, the streaming potential, V_s, is generated across the membrane, containing permselective channels in the presence of an osmotic gradient; its magnitude is related

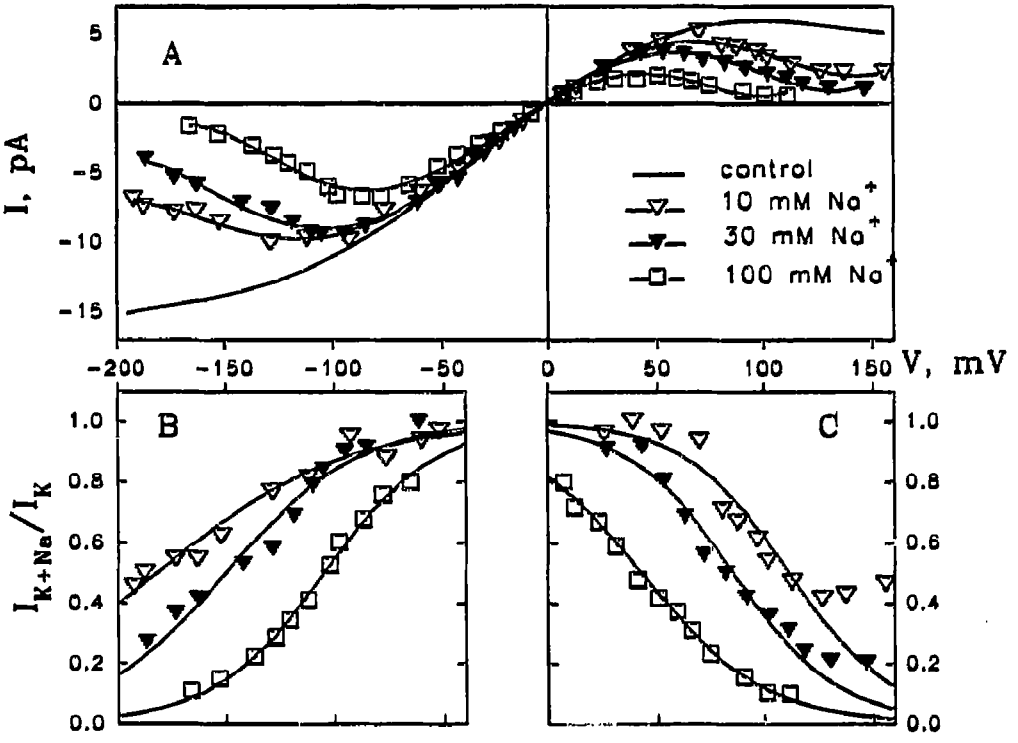

Fig. 2. (A) I–V relationships showing the blockade of open channel by Na^+. Control curve is low-order polynomial fitted to the data obtained from 5 cells. Voltage dependence of the external (B) and internal (C) Na^+ blockade. Solid curves represent the values of the fractional current (I_{K+B}/I_K) fitted to the data by Eq. (1). Symbols as in (A).

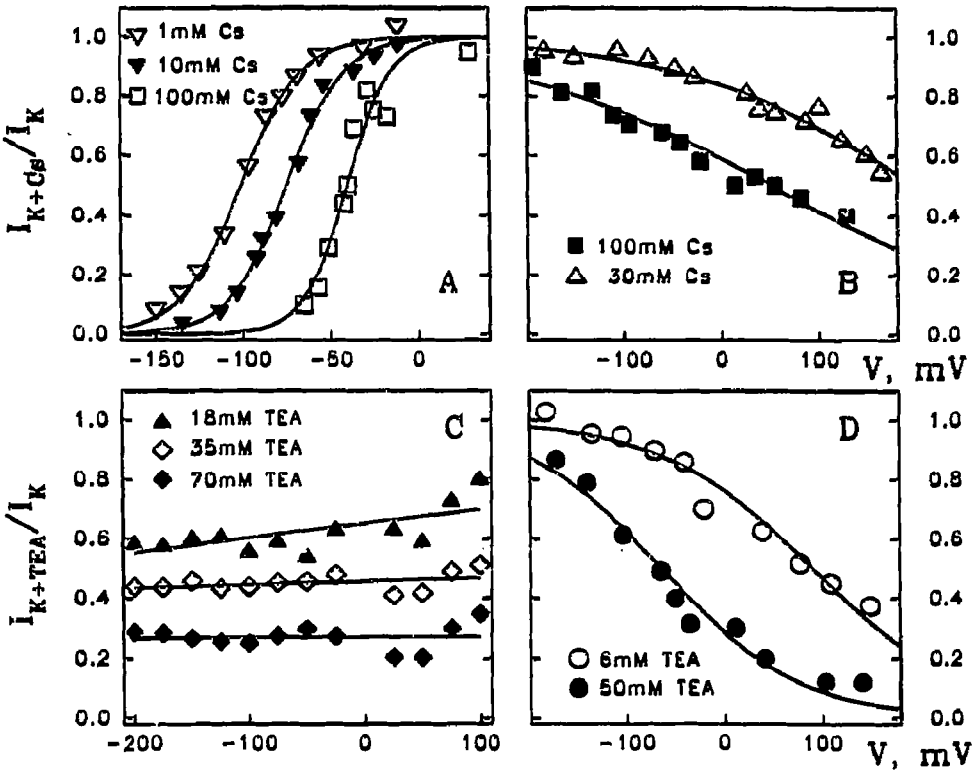

Fig. 3. Voltage dependence of external (A,C) and internal (B,D) channel blockade by Cs^+ (A,B) and TEA^+ (C,D). Solid curves are the best fits to the data by Eq. (1).

to the number of water molecules in the no-pass region, N by [9,12]:

$$V_s = N \Delta\pi \bar{V}_w / F \quad (2)$$

where $\Delta\pi$ is osmotic pressure difference across the membrane, $\bar{V}_w$ is the partial molar volume of water. At 20°C and with a 1 osmol gradient Eq. 2 reduces to $V_s = N \times 0.46$ (mV). Using this formula and the data in Fig. 4 it is calculated that the *Chara* K^+ channel contains up to 9 water molecules in the narrow region. Providing they form a linear row, it spans a distance of 28 Å.

4. DISCUSSION

Most K^+-selective channels are blocked in the open state by small monovalent cations such as Na^+, Cs^+ and TEA^+. These blockers are known to affect K^+ channels in plants, but the blocking parameters were not determined in most cases [13–15]. Although the present K^+ channel is thought to be of tonoplast origin [1,10] as compared with the K^+ channel in the plasma membrane of *Charophytes*, the blockade of both channels by external cations shows a common pattern (this study, [16,18]). Furthermore, the characteristics of ionic blockade for the *Chara* K^+ channel are very similar to those for the well-studied maxi-K_{Ca} and delayed rectifier (DR) K^+ channels in animals (Table I of this study, [6,8]). The latter channels, as the present one, have multi-ion pores, which is particularly evident in the peculiar voltage-dependent blockade by Na^+ and Cs^+ [7,19,21]. All three of the K^+ channels considered here have different internal and external TEA^+ receptors (wide mouths) located

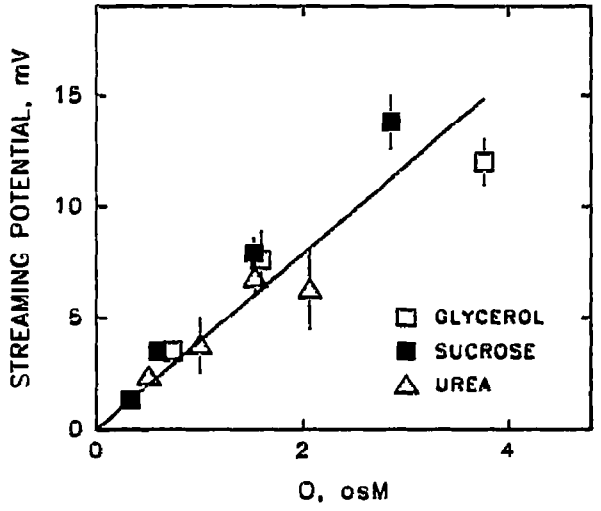

Fig. 4. Streaming potentials measured in different osmotic gradients established by the addition of urea (n=3), glycerol (n=2) or sucrose (n=4) into the bath. The straight line drawn through the points has a slope of 4.0 mV/Osmol, and N=9 according to Eq. (2).

at the sites which sensed only 0.0–0.35 of the applied electric field [8,20–21]. The external receptor in the *Chara* K^+ channel has much lower affinity for TEA^+ as compared with the DR and maxi-K_{Ca} channels, however, with the present K^+ channel, TEA^+ was more effective than its analogs of smaller (TMA^+) or of larger (TBA^+) size, similarly to that found with other channels [8].

The results of this paper support the view that the structure of the permeation pathway in K^+ channels is well-conserved; for instance, the maxi-K_{Ca} channel of bovine chromaffin cells and the *Chara* K^+ channel have very similar permeation characteristics, down to the details of conductance, selectivity and blockade [4,5,21]. The various K^+ channel types can be further classified by the length of their narrow regions (tunnels). The values of streaming potential measured in the *Chara* K^+ channel (this study), DR and maxi-K_{Ca} channels [9,22] are (in mV/osmol) 4.0, 4.5 and 0.9, respectively. Thus, it appears that the first two channels have a longer tunnel than the third one has. The longer tunnel seems plausible for electrostatic reasons to account for multi-ion occupancy. However, channels with a longer tunnel are expected to have lower conductance [6,8], and with *Chara* K^+ channel it is not the case. This can be explained if one assumes that the channel is actually made up of multiple low-conductance pores, gating in parallel; recent studies on the various K^+ channels argued in favour or this model [23–25].

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