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Unified modeling of conductance kinetics for low- and high-conductance potassium ion channels

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A kinetics model is proposed for the description of ion conductance of low- and high-conductance potassium ion channels. The model describes ion permeation through the selectivity filter, which is assumed to be the only conductance determining part of the open channel. The filter occupancy can vary from zero to three ions, affecting the ion entry and exit rates. Ion motion between the binding sites inside the filter is assumed fast compared to the latter rates allowing averaging the equilibrium entry and exit rate constants over the possible ion configurations in the filter with a particular occupancy. Averaged rate constants related to a pair of adjacent occupancy states characterize a particular ion permeation mechanism. An expression for the channel conductance as a function of the symmetrical external ion concentration is derived. It comprises a sum of concentration independent conductance amplitudes for different ion permeation mechanisms weighted by the equilibrium filter occupancy probabilities. It is shown that each amplitude (i.e., maximum contribution to the channel conductance from each conductance mechanism) is proportional to an averaged exit rate constant and to quantities characterizing the effect of the applied electric field on the rate constants and the equilibrium ion distribution in the filter. The conductance expression derived provides a good description of the experimentally observed conductance-concentration curves for low-conductance (e.g., Kir2.1) and high-conductance (e.g., KcsA) potassium channels. It enables one to obtain equilibrium ion binding constants at different filter occupancies and to calculate the average number of ions in the selectivity filter for a given external ion concentration. For KcsA this number (2.0 at 200 mM) is in a good agreement with the available experimental value (2.1 at 200 mM). For the high-conductance potassium channels the net negative electrical charge around the selectivity filter increases the ion binding constants, thereby causing the larger occupancy probabilities to occur at smaller external ion concentrations compared to the low-conductance channels. This substantially increases the contributions of the two- and three-ion permeation mechanisms, with the larger conductance amplitudes leading to increased channel conductance compared to the low-conductance channels.

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I. INTRODUCTION

Potassium ion channels are a family of pore-forming transmembrane proteins that selectively facilitate movements of potassium ions across the cell membranes. They play a key role in controlling membrane potential and modulating membrane excitations [1]. The conductive pathways of potassium channels are water filled pores where ions continue to have strong interactions with water molecules maintaining their hydration shells [2–5]. In the narrowest part of the channel, the selectivity filter, potassium ions make direct contacts with protein groups, being partially or fully dehydrated. The interactions with these groups (backbone carbonyl groups lining the filter), as well as the interactions with the charged groups in the vicinity of the filter, determine the selectivity and conductance properties of potassium channels [6–8].

The conductances of potassium channels differ from each other by orders of magnitude, ranging from 5–40 pS for low-conductance channels (e.g., the family of Kir channels) to 80–300 pS for high-conductance channels (e.g., KcsA, BK) [9–12]. This very large difference in conductances is especially intriguing in view of the highly conserved sequence Thr-Val-Gly-Tyr-Gly constituting the selectivity filter. The large range of conductances as well as the selectivity of potassium channels are among the issues which are currently unresolved. In this paper we present a kinetics model to account in a unified way for the conductance of the low- and high-conductance potassium channels, and we discuss differences in conduction mechanisms of these channels.

There have been numerous efforts to develop theoretical models for multi-ion channels which enable a simple analytical description of the concentration-dependent ion permeation kinetics in potassium channels. The simplest equation, initially developed for single-ion occupancy channels [13], but frequently used empirically for multi-ion potassium channels [1], is the Michaelis-Menten equation:

$$g(c) = g_{\max}\frac{c/c_{1/2}}{1 + c/c_{1/2}}. \tag{1}$$

Here c is the symmetrical concentration of conductive ions in the bathing solutions, $g_{\max}$ is the maximum (saturation) conductance of the channel and $c_{1/2}$ is the ion concentration at which $g = g_{\max}/2$ and which is related to the ion binding constant K_M by $K_M \equiv c_{1/2}^{-1}$.

To account for multiple ion occupancy effects in the selectivity filter of potassium channels, a single-vacancy model

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has been proposed [14,15]. In this model, the ions can occupy all n binding sites in the pore, or all but one site creating states with a single vacancy. Depopulation of the latter states at very high c leads to a decrease of internal ion transition rates, and as a result a decrease of the channel conductance. This model gives an additional c^2 term in the denominator of (1) which is proportional to the reciprocal rate constant for the ion transitions inside the channel [15].

Recently, a two state occupancy model (n and $n-1$ ions in the selectivity filter) has been proposed to describe the multiion permeation kinetics in KcsA or other structurally equivalent high-conductance channels [16,17]. This model is similar to the single-vacancy model, but the number of ions n is not necessarily equal to the number of ion binding sites. The ion translocation rate inside the selectivity filter is assumed to be fast, compared to the ion exit or entry rates. This assumption leads to an effective single-ion kinetics which is well described by the Michaelis-Menten equation.

The drawback of the last two models is that despite being multi-ion models, they are unable to describe multi-ion kinetics of high-conductance channels over a wide range of external ion concentration. Being limited to a description of only one permeation mechanism ($n \rightarrow n-1$) they do not take into account more complex nature of multi-ion kinetics in the selectivity filter of potassium channels, i.e., the possible coexistence of several ion permeation mechanisms, polarization effects, etc.

In a previous work [18] we proposed a kinetics model which considers more than two ion occupancy states in the selectivity filter, allowing coexistence of several ion permeation mechanisms. This model was shown to provide a better description of low-conductance potassium channels than the Michaelis-Menten model. However the limitations of the model (e.g., assumption of an occupancy-independent effective electrical mobility of ions in the selectivity filter region) reduce its validity to the low-conductance potassium channels.

The purpose of this work is to propose a unified theory for the conductance of the low- and high-conductance potassium channels and to derive more general expression for the channel conductance. This theory describes the permeation kinetics over a broad range of external ion concentration taking into account multiple occupancy states of the channel selectivity filter and the corresponding different conduction mechanisms.

II. STRUCTURE AND OCCUPANCY OF THE SELECTIVITY FILTER

Analysis of the primary sequences and proposed secondary structures of many potassium channels [1] and the determinations of the crystal structures of several of them (KcsA [3], MthK [19], KvAP [20], and KirBac1.1 [21]) have established a general view of the pore structure of most K channels [5]. In accordance with this view, the conductive pathway of a typical potassium channel consists of a water-filled cavity which can be wide open to the intracellular region in the conductive "open" state of the channel, and a rather narrow part, the selectivity filter, connecting the pore to the extracellular region. The filter as well as the whole channel protein has a fourfold symmetry and is made of residues with the conserved sequence motif TVGYG (Thr-Val-Gly-Tyr-Gly). The ion pathway in the filter is lined by the carbonyl oxygen atoms of the protein backbone and contains four K^+ ion binding sites, S_1-S_4 (starting from the extracellular end [3]). In each of these sites a potassium ion is coordinated by eight partially charged oxygen atoms [7] and can be partially or fully dehydrated.

In the linear-response regime (where the single-channel current I is proportional to the applied voltage V) the channel conductance g is determined primarily by the conductance of the selectivity filter. This is confirmed by experiments [11] where the substitution of the charged residues at the intracellular mouth of the channel (at the beginning of the water cavity) by neutral residues does not change the open channel conductance at low voltages. Therefore the channel permeation kinetics in the linear-response regime can be described by the kinetics of the selectivity filter only.

In many potassium channels (e.g., KcsA, Shaker, MthK, BK) the selectivity filter is surrounded by four Asp residues with negatively charged carboxyl groups situated at 6–8 Å from the ion permeation pathway. In this case the filter, of length approximately 12 Å, can accommodate up to three K^+ ions whose mutual repulsion is balanced by the interaction with these residues. A substantial contribution to the stability of the ions in the filter is made also by the interaction with the partial negative charges on the backbone carbonyl oxygen atoms.

The distribution of the ions among different binding sites inside the filter can be described by a set of states (ni) characterized by the probabilities p_{ni}. The first index $n=0,1,2,3$ corresponds to the number of ions occupying the filter (state occupancy number) and the second index $i=1,2,\ldots$ denotes the substates with the same occupancy number. It is assumed that the transitions between states, $(ni) \rightarrow (n'j)$, can be described by the rate constants $k_{ni,n'j}$. The rate constants corresponding to the transitions which change the occupancy number $(n' \neq n)$ are called entry or exit rate constants. To distinguish between the ion entry or exit from the different sides of the filter we use a superscript (L) or (R) in the notation for these rate constants (e.g., $k^{(L)}_{ni,n'j}$). A kinetics scheme of the selectivity filter showing the ion distribution states and corresponding transitions is depicted in Fig. 1. Compared to a previous work [18] describing the conductance kinetics of low-conductance potassium channels (e.g., Kir2.1), the present kinetics scheme contains additional three-ion states. These states probably do not make any noticeable contribution to the conductance of low-conductance channels with a net neutral charge around the selectivity filter, even at high external ion concentration. However, they may be necessary for the description of potassium channels with a net negative charge around the selectivity filter, e.g., KcsA. The results of the free energy surface calculations for the three-ion configurations of KcsA [22] show that some of these three-ion states of the filter are stable, i.e., there is a small barrier of about 1 kcal/mol for the third ion to exit the filter, and, hence, their occupancy probabilities are not negligible. The fact that not all of the

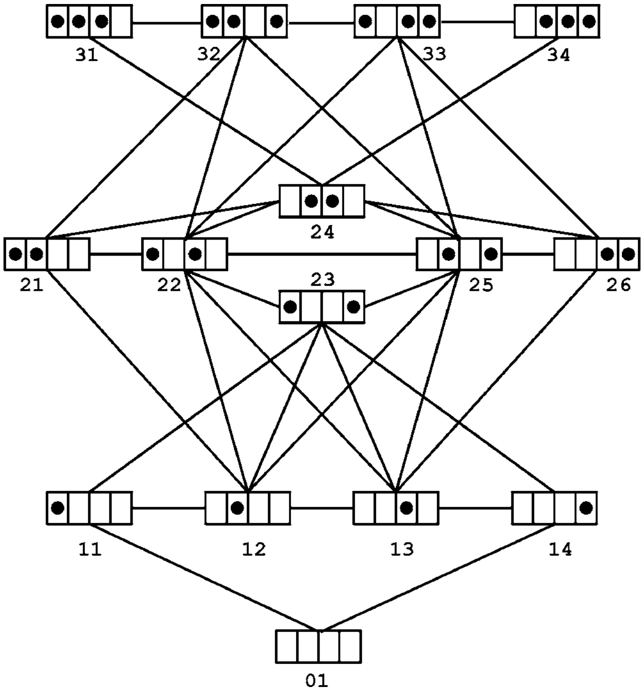

FIG. 1. Kinetics scheme for the potassium channel selectivity filter.

three-ion states are stable suggests that the four-ion state of the filter will be unstable and therefore can be ignored for all potassium channels.

Molecular dynamics (MD) and Brownian dynamics (BD) simulation results for the KcsA [22–25] and the KirBac1.1 [26] channels suggest that at equilibrium or at small steady-state currents the ion motion inside the selectivity filter $[(ni)\rightarrow(nj)$ transitions$]$ occurs on a nanosecond time scale, which is much faster than the average time between ion exit or entry events (tens of nanoseconds at physiological voltage and ion concentration) even for the most conducting channels. As shown in Ref. [22] for the KcsA channel, fast motion of two ions inside the filter is caused by a small barrier of approximately $2k_BT$ between two most probable two-ion configurations: substates (22) and (25) in Fig. 1.

Such a significant separation of the ion motion time scales implies that after a change of occupancy number n the relaxation of the ion distribution inside the filter, which occurs on the same time scale as the ion motion in the filter, is fast compared to the ion entry-exit time scale. Therefore, the influence of the ion entry-exit events on the equilibrium ion distribution inside the filter corresponding to a given occupancy number is assumed negligible. It also means that the relative probabilities of the substates with the same occupancy number do not depend on the external ion concentration c. These probabilities are the conditional probabilities $p_i^{(n)}$ to find the selectivity filter in the substate i, given the prescribed value of the occupancy number n, i.e.,

$$p_i^{(n)} = p_{ni}/p_n, \tag{2}$$

where

$$p_n = \sum_j p_{nj} \tag{3}$$

is the occupancy probability, i.e., the probability to have n ions in the selectivity filter irrespective of substate j.

The probabilities p_n are concentration-dependent quantities which determine the average occupancy number $\bar{n}(c)$,

$$\bar{n}(c) = \sum_{n=0}^{3} n p_n(c). \tag{4}$$

The fast relaxation of the ion distribution and the concentration independence of $p_i^{(n)}$ allow us to carry out an averaging of the entry and exit rate constants $k_{ni,n'j}$ over the initial states (ni) and a summing over the final states $(n'j)$, thereby introducing the reduced entry and exit rate constants $k_{n,n'}^{(L)}$ and $k_{n,n'}^{(R)}$ [18]

$$k_{n,n'}^{(L)} = \sum_{\{ij\}} p_i^{(n)} k_{ni,n'j}^{(L)}. \tag{5}$$

Here, $\Sigma_{\{ij\}}$ denotes a sum over those pairs of states $(ni,n'j)$ which correspond to the ion entry (or exit) from the L side of the filter. A similar expression holds for $k_{n,n'}^{(R)}$.

Defining equilibrium constants K_n,

$$K_n \equiv k_{n-1,n}/k_{n,n-1}, \tag{6}$$

where

$$k_{n,n'} \equiv k_{n,n'}^{(L)} + k_{n,n'}^{(R)}, \tag{7}$$

one can show [18] that the equilibrium value of $p_n(c)$ is given by the equation

$$p_n(c) = \frac{c^n \prod_{n'=0}^{n} K_{n'}}{1 + \sum_{m=1}^{3} c^m \prod_{n'=1}^{m} K_{n'}}, \tag{8}$$

where $K_0 \equiv 1$. To get (8), an implicit boundary condition for $p_n(c)$ was used in Ref. [18], $p_n(0) = \delta_{n,0}$. Another assumption used is that the ion entry rates are linear in the bulk ion concentration c. This implies that the local ion concentration near both ends of the filter is linear in c, which is not obviously true if channel protein and membrane surface charges are involved.

III. MECHANISMS OF CONDUCTANCE AND ION PERMEATION KINETICS

Consider a voltage V applied across the membrane where the potassium ion channel is embedded. It was shown in Ref. [24], that for a wide open potassium channel almost all of the electrical potential difference falls across the selectivity filter, where it is a linear function of distance. Therefore, we can consider V to be applied across the selectivity filter of the conducting channel. We also assume that the symmetrical solutions at both sides of the membrane contain no other ions (except potassium) capable of permeating the channel.

At a small voltage ($V \ll k_B T/q$) we can expand the current I across the filter as

$$I(V) = I(0) + \left(\frac{dI}{dV}\right)_{V=0} V + \frac{1}{2}\left(\frac{d^2 I}{dV^2}\right)_{V=0} V^2 + \cdots . \qquad (9)$$

Here k_B is Boltzmann's constant, T the temperature and q the charge of a permeating ion. In symmetrical ion solutions we have $I(0)=0$ and in linear approximation (Ohm's law) we therefore have

$$I(V) = \left(\frac{dI}{dV}\right)_{V=0} V \equiv gV, \qquad (10)$$

where $g \equiv (dI/dV)_{V=0}$ is the conductance of the channel.

One of the possible mechanisms of ion conduction, which has been presumed to be the main mechanism for the low-conductance potassium channels [18] and was implicitly assumed in Refs. [16,17], involves the change of the number of ions in the filter on the time scale larger than time scale for the ion motion inside the filter $[(ni) \rightarrow (nj)$ transitions$]$. For this mechanism, the relaxation to equilibrium of the ion distribution in the filter after an ion entry to or exit from the filter is assumed to be fast and the only rate limiting steps of ion permeation through the filter are ion entry-exit events. This allows us to neglect the details of ion motion inside the filter and to reduce the number of quantities involved in the description of steady-state concentration dependent ion current. The independent quantities now are the reduced entry-exit rate constants $k_{n,n'}$ (7) which fully determine the equilibrium constants K_n (6) and the occupancy probabilities $p_n(c)$ (8). The voltage dependences of the reduced rate constants and the occupancy probabilities describe the ion current through the channel. Our goal is to derive an expression for the conductance g as a function of the equilibrium values of these quantities.

At small applied voltage V (small electrical field inside the filter) there are small changes in rate constants characterizing the transitions between states of the selectivity filter. The changes in the rate constants describing the ion motion inside the filter, $k_{ni,nj}$, cause deviations of the probabilities $p_i^{(n)}$ and p_{ni} from their equilibrium values (at $V=0$). These deviations can be considered as a polarization of the equilibrium ion distribution in the filter and, according to (5), contribute to the changes of the reduced entry-exit rate constants $k_{n,n'}^{(L,R)}$. It is interesting to note that with fast relaxation of the filter ion distribution, the voltage induced changes in the entry-exit rate constants $k_{ni,n'j}^{(L,R)}$ do not affect the relative probabilities $p_i^{(n)}$ and thus have only direct effect on $k_{n,n'}^{(L,R)}$.

In addition to the mechanism for ion conduction discussed above, a different kind of ion transfer through the filter is possible. During this transfer the energy of an entering ion, reaching the top of the first energy barrier in the filter, is not fully dissipated during the following motion to the first binding site inside the filter, but (through ion-ion interaction) is transferred to the other ions in the filter to overcome the other energy barriers thus enabling them to exit the filter. This kind of ion motion through the filter is considered a major conduction mechanism in high-conductance potassium channels at physiological ion concentrations, such as the KcsA [6,27], and is called the "throughput" (or "knock-on") ion conduction mechanism.

The rate of the "throughput" mechanism can be characterized by the probability per unit time for an entering ion to reach the top of the first energy barrier in the filter, i.e., to reach the transition state, together with the probability that this ion will move "forward" and the transition state will "decay" through the exit of the most distant ion from the opposite end of the filter (by analogy with chemical rate theory we can denote this probability the "transmission" probability). The alternatives to this evolution of the transition state are "backward" motion of the entering ion (i.e., reflection at the barrier top), or motion of this ion to the nearest binding site inside the filter with dissipation of its energy and without another ion exiting the filter.

Combining the probability to reach the transition state of the filter which is favorable for the "throughput" ion motion and the "transmission" probability of the "forward" evolution of this transition state in one voltage dependent rate constant, $k^{(*)}$, we can define the rate of the unidirectional (e.g., left-to-right) "throughput" ion transfer as a product: $c \times k_{ni}^{(*)} \times p_{ni}^{(*)}$, where $p_{ni}^{(*)}$ is the probability of the state which precedes the transition state of the filter that is favorable for the "forward" dissipationless ion transfer. As in the case with the entry rate constants, this equation implies linear behavior of the local ion concentrations near the entrances of the filter with bulk potassium ion concentration c.

It is worth noting here that the full rate of the unidirectional "throughput" ion transfer is the sum of the above product over all possible states (ni) which can precede the transition states that are favorable for the subsequent dissipationless ion transfer. However, the experimentally observed ion distribution in the KcsA filter and the geometry of the filter have lead to the suggestion [6,27] that only one doubly occupied ($n=2$) state for each direction of ion transfer is suitable for the "throughput" ion transitions. Following this suggestion, we denote the probabilities of these two states [states (25) and (22) in the Fig. 1] as $p_t^{(L)}$ and $p_t^{(R)}$ and the corresponding rate constants as $k_t^{(L)}$ and $k_t^{(R)}$ for the left-to-right and right-to-left "throughput" ion transfers, respectively [omitting the state indices ni and sign $(*)$].

The ion current through the filter is the difference of left-to-right and right-to-left ion fluxes. At small applied voltage, these fluxes are not equal due to voltage induced changes in the rate constants as compared to their equilibrium values. Using the definition of the reduced entry and exit rate constants $k_{n,n'}^{(L,R)}$ (5) and taking into account the above discussed two mechanisms of ion transfer, a symmetrical (with respect to left and right entry barriers) expression for the ion current through the selectivity filter can be written as

$$I(V) = \frac{q}{2}\Bigg(\sum_{n=0}^{2} [k_{n,n+1}^{(L)}(V) - k_{n,n+1}^{(R)}(V)] p_n(V) c - \sum_{n=0}^{2} [k_{n+1,n}^{(L)}(V) - k_{n+1,n}^{(R)}(V)] p_{n+1}(V) + 2[k_t^{(L)}(V) p_t^{(L)}(V) c - k_t^{(R)}(V) p_t^{(R)}(V) c]\Bigg). \qquad (11)$$

To derive the equation for the conductance, which, according to definition (10), is the voltage derivative of the ion current (11), we need to obtain V derivatives of the probabilities $p_n(V)$ and $p_t^{(L,R)}(V)$, the "throughput" rate constants $k_t^{(L,R)}(V)$ and the reduced entry and exit rate constants $k_{n,n'}^{(L,R)}(V)$. The voltage dependence of the latter is determined through the rate constants $k_{ni,n'j}^{(L,R)}(V)$ and the relative probabilities $p_i^{(n)}(V)$ [see Eq. (5)]. Those readers not interested in the details of the derivation can proceed directly to Eq. (39).

Using the standard assumption [1] of exponential dependence of the rate constants on applied voltage we write

$$k_{ni,n'j}^{(L,R)}(V) = k_{ni,n'j}^{(L,R)} \exp\left(\frac{\pm \delta_{ni,n'j}^{(L,R)} Vq}{k_B T} \right), \tag{12}$$

where $\delta_{ni,n'j}^{(L,R)}$ is the dimensionless apparent electrical distance for the ion transition $(ni) \rightarrow (n'j)$. [The sign preceding $\delta_{ni,n'j}^{(L,R)}$ in (12) depends on the direction of the ion transition with respect to the direction of the applied field, i.e., $+\delta$ corresponds to the ion transfer along the field direction.]

The effective "throughput" rate constants can be written as

$$k_t^{(L,R)}(V) = \kappa_0 \left(1 \pm \frac{\delta_\kappa^{(L,R)} Vq}{k_B T} \right) k_{t,\mathrm{TST}}^{(L,R)} \exp\left(\frac{\pm \delta_t^{(L,R)} Vq}{k_B T} \right). \tag{13}$$

Here, we write the rate constant as a product of a transition state theory (TST) rate constant $k_{t,\mathrm{TST}}^{(L,R)}(V)$ with its exponential dependence on applied voltage characterized by the parameter $\delta_t^{(L,R)}$ (equivalent to the apparent electrical distance $\delta_{2i,3j}^{(L,R)}$ where $i=t$) and an effective transmission coefficient $\kappa(V)$ with a linear voltage dependence characterized by the parameter $\delta_\kappa^{(L,R)}$.

Voltage derivatives of (12) and (13) are straightforward and we shall not write them here separately.

Next, we need to analyze the voltage dependences of the probabilities p_n and $p_i^{(n)}$. Since, using (2), the terms with the reduced rate constants in (11) can be rewritten as

$$p_n(V) k_{n,n'}^{(L,R)}(V) = \sum_{\{ij\}} p_{ni}(V) k_{ni,n'j}^{(L,R)}(V), \tag{14}$$

we can proceed with the voltage dependences of the state probabilities $p_{ni}(V)$.

To determine the voltage derivative of $p_{ni}(V)$, we first write the rate equations for $p_{nj}(V)$; with the notation $\dot{p}_{nj}(V) \equiv dp_{nj}(V)/dt$, we have

$$\dot{p}_{nj}(V) = \sum_{i \neq j} p_{ni}(V) k_{ni,nj}(V) - \sum_{i \neq j} p_{nj}(V) k_{nj,ni}(V). \tag{15}$$

Here, taking into account the different time scales of the fast internal ion transitions and the slow ion exit-entry rates, we neglect the small contribution from the possible net ion flux across the filter boundary to and from the state (nj). In other words, we assume that at small voltage ion exit-entry events have little or no effect on the voltage induced changes of ion distribution.

Denoting the deviations of $p_{ni}(V)$ and $k_{ni,nj}(V)$ from their equilibrium values (at $V=0$) as $\Delta p_{ni}(V)$ and $\Delta k_{ni,nj}(V)$, respectively (from here on, for the equilibrium quantities we will use the notations without a functional dependence on V or without symbol Δ, e.g., p_{ni} or $k_{ni,nj}$), and taking into account that at equilibrium the left- and right-hand sides of (15) are equal to zero, we can rewrite (15) in the form

$$\dot{p}_{nj}(V) = \sum_{i \neq j} [p_{ni} \Delta k_{ni,nj}(V) + \Delta p_{ni}(V) k_{ni,nj}] - \sum_{i \neq j} [p_{nj} \Delta k_{nj,ni}(V) + \Delta p_{nj}(V) k_{nj,ni}]. \tag{16}$$

Since we are interested here only in a linear response at small V, we neglect the terms quadratic in V, i.e., $\Delta p \Delta k$ terms.

At small constant V, after the relaxation of the ion distribution to its voltage induced polarized state, the left-hand side of (16) is equal to zero. Dividing the right-hand side of (16) by the voltage change ΔV ($\Delta V \equiv V$) we get

$$\frac{\Delta p_{nj}}{\Delta V} \sum_{i \neq j} k_{nj,ni} = -p_{nj} \sum_{i \neq j} \frac{\Delta k_{nj,ni}}{\Delta V} + \sum_{i \neq j} \left(p_{ni} \frac{\Delta k_{ni,nj}}{\Delta V} + \frac{\Delta p_{ni}}{\Delta V} k_{ni,nj} \right). \tag{17}$$

In the limit of very small ΔV, replacing the ratios of the changes $(\Delta \cdots)$ by the voltage derivatives at $V=0$ and using the assumption of exponential dependence of the rate constants $k_{ni,nj}$ on applied voltage [similar to the dependence of the entry-exit rate constants (12)], we have

$$\frac{dp_{nj}}{dV} \sum_{i \neq j} k_{nj,ni} = -p_{nj} \sum_{i \neq j} \frac{q \delta_{nj,ni}}{k_B T} k_{nj,ni} + \sum_{i \neq j} \left(p_{ni} \frac{q \delta_{ni,nj}}{k_B T} k_{ni,nj} + \frac{dp_{ni}}{dV} k_{ni,nj} \right). \tag{18}$$

Assuming the apparent electrical distances $\delta_{nj,ni}$ for the ion transitions inside the selectivity filter do not depend on the details of the ion distribution for initial and final substates, i.e., have the same magnitude δ_0 and differ only in sign, $\delta_{nj,ni} = \pm \delta_0 = s_{nj,ni} \delta_0$ (the sign, $s_{nj,ni} = \pm 1$, depends on the direction of the ion transition with respect to the direction of the applied electrical field and is "+" for $j < i$ in the case of left-to-right field), and taking into account the condition of detailed balance for the transitions between two states at equilibrium,

$$p_{ni} k_{ni,nj} = p_{nj} k_{nj,ni}, \tag{19}$$

we can rewrite (18) as

$$\frac{dp_{nj}}{dV} \sum_{i \neq j} k_{nj,ni} = -\frac{2q\delta_0}{k_B T} p_{nj} \sum_{i \neq j} k_{nj,ni} s_{nj,ni} + \sum_{i \neq j} \frac{dp_{ni}}{dV} k_{ni,nj}. \tag{20}$$

Since $p_{nj} \Sigma_i k_{nj,ni}$ is the sum of the equilibrium fluxes originating from the state (nj), the quantity $p_{nj} \Sigma_i k_{nj,ni} s_{nj,ni}$ is the difference of the equilibrium fluxes in the left and right directions originating from this state. Denoting this equilibrium quantity as

$$f_{nj} \equiv p_{nj}\sum_{i\neq j} k_{nj,ni}s_{nj,ni}, \qquad (21)$$

and introducing the abbreviated notation for the voltage derivative of $p_{nj}(V)$ at $V=0$, i.e., p'_{nj}, we can rewrite (20) in the form

$$p'_{nj}\sum_{i\neq j} k_{nj,ni} = -\frac{2q\delta_0}{k_BT}f_{nj} + \sum_{i\neq j} p'_{ni}k_{ni,nj}. \qquad (22)$$

This equation can be easily transformed to the following matrix equation:

$$P_nR_n = \left(\frac{2q\delta_0}{k_BT}\right)F_n. \qquad (23)$$

Here, the elements of the row matrices P_n and F_n are p'_{ni} and f_{nj}, respectively, and the square matrix $R_n=\{r_{ni,nj}\}$ is determined by the matrix equation

$$\{r_{ni,nj}\} = \{k_{ni,nj}\} - \{\delta_{ij}\}\sum_{l} k_{nj,nl}, \qquad (24)$$

where $\{\delta_{ij}\}$ is a diagonal unit matrix and $k_{ni,nj}=0$ for $i=j$ and for all not allowed ion transitions in the selectivity filter.

The quantities p'_{ni} characterize the susceptibility of the state (ni) populations to deviate from their equilibrium values p_{ni} under the small applied electrical field, i.e., $\Delta p_{ni} = p'_{ni}\Delta V$. Accordingly, these deviations and the matrix P_n can be considered as the "polarization" and the electrical "susceptibility" of the ion distribution in the filter.

In addition to (23) the quantities p'_{ni} should satisfy the normalization equation

$$\sum_{i} p'_{ni} = 0, \qquad (25)$$

which follows from the definition (3) and the approximations used for (15). Combined, Eqs. (23) and (25) fully determine the quantities p'_{ni}.

Now we can write down the voltage derivatives for the different terms of the expression for the ion current (11). For the first two terms of the right-hand side of (11) connected with the ion entries from "left" and "right" sides of the filter (the $n\rightarrow n+1$ transitions) these derivatives are

$$\left(\frac{dp_n(V)k^{(L)}_{n,n+1}(V)}{dV}\right)_{V=0} = \sum_{\{ij\}}\left[p'_{ni}k^{(L)}_{ni,n+1j} + \left(\frac{\delta^{(L)}_{ni,n+1j}q}{k_BT}\right)p_{ni}k^{(L)}_{ni,n+1j}\right]. \qquad (26)$$

Assuming $\delta^{(L,R)}_{ni,n'j}=\delta^{(L,R)}_{n,n'}$ (i.e., $\delta^{(L,R)}_{ni,n'j}$, similar to the electrical distances for the internal ion transitions $\delta_{ni,nj}$, do not depend on the details of ion distribution for the initial and final substates), we can rewrite (26) as

$$\left(\frac{dp_n(V)k^{(L)}_{n,n+1}(V)}{dV}\right)_{V=0} = \frac{p_nq}{k_BT}\left[\delta_0\sum_{\{ij\}} p'_{(n)i}k^{(L)}_{ni,n+1j} + \delta^{(L)}_{n,n+1}\sum_{\{ij\}} p^{(n)}_i k^{(L)}_{ni,n+1j}\right]. \qquad (27)$$

Here, in analogy to the relative probability $p^{(n)}_i$, we introduce the quantity,

$$p'_{(n)i} = \frac{p'_{ni}}{p_n}\left(\frac{q\delta_0}{k_BT}\right)^{-1}, \qquad (28)$$

which describes the relative electrical "susceptibility" of the ion distribution independent of the filter occupancy probability $p_n(c)$.

Let us denote the first sum on the right-hand side of (27) as

$$k^{(L)\ \mathrm{pol}}_{n,n+1} = \sum_{\{ij\}} p'_{(n)i}k^{(L)}_{ni,n+1j}. \qquad (29)$$

We note that the quantity $cp_nk^{(L)\ \mathrm{pol}}_{n,n+1}$ is the contribution to the change of the unidirectional ion flux per unit voltage drop across the left entry barrier related to the polarization of the ion distribution in the filter. Therefore, in analogy to the reduced rate constants (5), we can call $k^{(L,R)\ \mathrm{pol}}_{n,n'}$ and related ion fluxes the reduced "polarization" rate constants and "polarization" ion fluxes, respectively. Since the sign of $p'_{(n)i}$ can be positive or negative, the value of $k^{(L,R)\ \mathrm{pol}}_{n,n'}$ can also be positive or negative, in contrast to positive values of the reduced rate constants $k^{(L,R)}_{n,n'}$. Thus, the "polarization" ion flux could make a positive or negative contribution to the unidirectional ion flux through the entry filter barrier.

Now (27) can be rewritten as

$$\left(\frac{dp_n(V)k^{(L)}_{n,n+1}(V)}{dV}\right)_{V=0} = \frac{p_nq}{k_BT}\left(\delta_0k^{(L)\ \mathrm{pol}}_{n,n+1} + \delta^{(L)}_{n,n+1}k^{(L)}_{n,n+1}\right). \qquad (30)$$

This equation clearly shows the two major contributors to the change of the flux per unit voltage as discussed above: the "polarization" flux and the flux connected with the voltage-dependent change of entry-exit rate constants. This last flux is proportional to the equilibrium unidirectional flux $cp_nk^{(L)}_{n,n+1}$.

A similar expression can be written for the $n+1\rightarrow n$ ion transitions [the next two terms on the right-hand side of (11) connected with the outgoing ion fluxes from the filter]. In this case, however, the sign preceding $\delta^{(L)}_{n+1,n}$ will be opposite to the sign preceding $\delta^{(L)}_{n,n+1}$ [if we choose both quantities to be positive]. Thus, we have

$$\left(\frac{dp_{n+1}(V)k^{(L)}_{n+1,n}(V)}{dV}\right)_{V=0} = \frac{p_{n+1}q}{k_BT}\left(\delta_0k^{(L)\ \mathrm{pol}}_{n+1,n} - \delta^{(L)}_{n+1,n}k^{(L)}_{n+1,n}\right). \qquad (31)$$

The sign preceding δ_0 does not change since the signs of the "polarization" parameters p'_{ni} do not depend on the direction of the ion transition and are determined by the direction of the electric field only.

Expressions equivalent to (30) and (31) can be written for the ion transitions through the right entry barrier. The signs preceding $\delta^{(R)}_{n,n+1}$ and $\delta^{(R)}_{n+1,n}$ are again prescribed according to the direction of the ion transition (compared to the direction of the applied electric field).

The remaining voltage derivatives to determine are for the part of (11) describing the "throughput" mechanism of ion permeation and can be obtained from (13),

$$\left(\frac{dk_t^{(L)}(V)p_t^{(L)}(V)}{dV}\right)_{V=0} = \left(\frac{p_2 q}{k_B T}\right)\kappa_0 k^{(L)}_{t,\mathrm{TST}}[(\delta^{(L)}_\kappa + \delta^{(L)}_t)p^{(2)}_{(L)t} + \delta_0 p'^{(L)}_{(2)t}]. \tag{32}$$

Here $p^{(2)}_{(L)t}$ is defined as $p^{(L)}_t/p_2$.

Substituting these expressions into the voltage derivative of (11), and using the definition of the conductance (10), we have

$$\begin{aligned}g = \frac{q^2}{2k_BT}\Bigg(&\sum_{n=0}^{2}[cp_n(c)(\delta_0 k^{(L)\ \mathrm{pol}}_{n,n+1} + \delta^{(L)}_{n,n+1}k^{(L)}_{n,n+1})\\ &- p_{n+1}(c)(\delta_0 k^{(L)\ \mathrm{pol}}_{n+1,n} - \delta^{(L)}_{n+1,n}k^{(L)}_{n+1,n}) - cp_n(c)(\delta_0 k^{(R)\ \mathrm{pol}}_{n,n+1}\\ &- \delta^{(R)}_{n,n+1}k^{(R)}_{n,n+1}) + p_{n+1}(c)(\delta_0 k^{(R)\ \mathrm{pol}}_{n+1,n} + \delta^{(R)}_{n+1,n}k^{(R)}_{n+1,n})]\\ &+ 2cp_2(c)\kappa_0\{k^{(L)}_{t,\mathrm{TST}}[(\delta^{(L)}_\kappa + \delta^{(L)}_t)p^{(2)}_{(L)t} + \delta_0 p'^{(L)}_{(2)t}]\\ &- k^{(R)}_{t,\mathrm{TST}}[(-\delta^{(R)}_\kappa - \delta^{(R)}_t)p^{(2)}_{(R)t} + \delta_0 p'^{(R)}_{(2)t}]\}\Bigg).\end{aligned} \tag{33}$$

Using the definitions of equilibrium constants (6) and (7), and using the relation for equilibrium occupancy probabilities,

$$p_{n+1} = K_{n+1}cp_n = \frac{k_{n,n+1}}{k_{n+1,n}}cp_n, \tag{34}$$

we can rewrite (33),

$$\begin{aligned}g = \frac{q^2}{2k_BT}\Bigg[&\sum_{n=0}^{2}p_{n+1}\Bigg(\frac{k^{(L)}_{n+1,n}+k^{(R)}_{n+1,n}}{k^{(L)}_{n,n+1}+k^{(R)}_{n,n+1}}(\delta_0 k^{(L)\ \mathrm{pol}}_{n,n+1} - \delta_0 k^{(R)\ \mathrm{pol}}_{n,n+1}\\ &+ \delta^{(L)}_{n,n+1}k^{(L)}_{n,n+1} + \delta^{(R)}_{n,n+1}k^{(R)}_{n,n+1}) - \delta_0 k^{(L)\ \mathrm{pol}}_{n+1,n} + \delta_0 k^{(R)\ \mathrm{pol}}_{n+1,n}\\ &+ \delta^{(L)}_{n+1,n}k^{(L)}_{n+1,n} + \delta^{(R)}_{n+1,n}k^{(R)}_{n+1,n}\Bigg) + 2\frac{k_{32}}{k_{23}}p_3\kappa_0\{k^{(L)}_{t,\mathrm{TST}}[(\delta^{(L)}_\kappa\\ &+ \delta^{(L)}_t)p^{(2)}_{(L)t} + \delta_0 p'^{(L)}_{(2)t}] + k^{(R)}_{t,\mathrm{TST}}[(\delta^{(R)}_\kappa + \delta^{(R)}_t)p^{(2)}_{(R)t}\\ &- \delta_0 p'^{(R)}_{(2)t}]\}\Bigg].\end{aligned} \tag{35}$$

Assuming left-right symmetry for the entry and exit apparent electrical distances $\delta^{(L,R)}_{n,n'}$ and $\delta^{(L,R)}_t$ and for the parameter $\delta^{(L,R)}_\kappa$, we can simplify (35) to the form

$$\begin{aligned}g = \frac{q^2}{2k_BT}\Bigg[&\sum_{n=0}^{2}p_{n+1}\Bigg(k_{n+1,n}(\delta_{n,n+1} + \delta_{n+1,n}) + \frac{k_{n+1,n}}{k_{n,n+1}}\delta_0(k^{(L)\ \mathrm{pol}}_{n,n+1}\\ &- k^{(R)\ \mathrm{pol}}_{n,n+1}) + \delta_0(-k^{(L)\ \mathrm{pol}}_{n+1,n} + k^{(R)\ \mathrm{pol}}_{n+1,n})\Bigg) + 2\frac{k_{32}}{k_{23}}p_3[(\delta_\kappa + \delta_t)\widetilde{k}_t\\ &+ \delta_0(k^{(L)\ \mathrm{pol}}_t - k^{(R)\ \mathrm{pol}}_t)]\Bigg],\end{aligned} \tag{36}$$

where

$$\widetilde{k}_t = \kappa_0(k^{(L)}_{t,\mathrm{TST}}p^{(2)}_{(L)t} + k^{(R)}_{t,\mathrm{TST}}p^{(2)}_{(R)t}) \tag{37}$$

is the reduced "throughput" rate constant, and

$$k^{(L,R)\ \mathrm{pol}}_t = \kappa_0 k^{(L,R)}_{t,\mathrm{TST}}p'^{(L,R)}_{(2)t} \tag{38}$$

is the reduced "polarization" throughput rate constant.

Equation (36) can be further simplified by grouping similar terms

$$\begin{aligned}g(c) = \frac{q^2}{2k_BT}[&k_{10}A_1p_1(c) + k_{21}A_2p_2(c) + k_{32}A_3p_3(c)\\ &+ k_{32}A_{tp}p_3(c)],\end{aligned} \tag{39}$$

where the coefficients A_n and A_{tp} are

$$A_1 = \delta_{01} + \delta_{10} + \delta_0\left(\frac{-k^{(L)\ \mathrm{pol}}_{10} + k^{(R)\ \mathrm{pol}}_{10}}{k_{10}}\right), \tag{40}$$

$$A_2 = \delta_{12} + \delta_{21} + \delta_0\left(\frac{k^{(L)\ \mathrm{pol}}_{12} - k^{(R)\ \mathrm{pol}}_{12}}{k_{12}} + \frac{-k^{(L)\ \mathrm{pol}}_{21} + k^{(R)\ \mathrm{pol}}_{21}}{k_{21}}\right), \tag{41}$$

$$A_3 = \delta_{23} + \delta_{32} + \delta_0\left(\frac{k^{(L)\ \mathrm{pol}}_{23} - k^{(R)\ \mathrm{pol}}_{23}}{k_{23}} + \frac{-k^{(L)\ \mathrm{pol}}_{32} + k^{(R)\ \mathrm{pol}}_{32}}{k_{32}}\right), \tag{42}$$

$$A_{tp} = 2\left((\delta_\kappa + \delta_t)\frac{\widetilde{k}_t}{k_{23}} + \delta_0\frac{k^{(L)\ \mathrm{pol}}_t - k^{(R)\ \mathrm{pol}}_t}{k_{23}}\right). \tag{43}$$

The quantities A_n are essentially the sums of the apparent electrical distances (entry $\delta_{n-1,n}$, exit $\delta_{n,n-1}$, and internal δ_0). The internal apparent electrical distance δ_0 is weighted by the ratios of the reduced "polarization" rate constants and the reduced entry and exit rate constants. Since all of these quantities do not depend on the external ion concentration, the A_n can be considered as c-independent parameters in (39). The same is true for A_{tp} which is the sum of weighted "throughput" $(\delta_\kappa + \delta_t)$ and internal δ_0 apparent electrical distances.

It is interesting to note that the weight following δ_0 in (43) is fully equivalent to the first term of the corresponding weight in (42) which is the ratio of the entry "polarization" and entry reduced rate constants. The weight following the "throughput" electrical distances is the ratio of the reduced "throughput" $\widetilde{k}_t$ and entry k_{23} rate constants which is equivalent to the ratio of the realization probabilities for the "throughput" and three-ion conduction mechanisms.

IV. RESULTS

Equation (39) describes in a simple manner the dependence of the potassium ion channel conductance on the symmetrical external ion concentration. The derivation of this equation is based on the formal kinetic description of the contributions of four possible ion permeation mechanisms to the ion current caused by a small applied voltage. Each of these mechanisms involves a different combination of the initial and final numbers of ions in the selectivity filter for the ion permeation event (an ion crosses the filter entry barrier) on the time-scale larger than the relaxation times for the ion distribution in the filter and corresponds to one term in (39). The concentration dependence of each term is determined by the dependence of the equilibrium occupancy probabilities $p_n(c)$ given by (8) with n corresponding to the largest number of ions involved in each permeation mechanism. Denoting the coefficients of $p_n(c)$ in (39) as g_n and g_{tp}, we can rewrite this equation as

$$g(c) = g_1 p_1(c) + g_2 p_2(c) + g_3 p_3(c) + g_{tp} p_3(c), \tag{44}$$

where

$$g_n = \frac{q^2}{2k_B T} k_{nn-1} A_n \tag{45}$$

and

$$g_{tp} = \frac{q^2}{2k_B T} k_{32} A_{tp}. \tag{46}$$

Parameters g_n and g_{tp}, which by definition are c-independent quantities, can be considered as the amplitudes of the contributions of different ion permeation mechanisms to the total channel conductance, i.e., the maximum channel conductance each mechanism can provide. According to (45) and (46) they are proportional to the reduced exit rate constants which are sensitive to the equilibrium ion distribution and degree of ion-ion interaction in the selectivity filter. They are also proportional to the coefficients A_n and A_{tp} which depend on the apparent electrical distances and the polarization properties of the equilibrium ion distribution described by the matrices P_n and related reduced "polarization" rate constants (29) and (38).

Probabilities $p_n(c)$ in (44) can be considered as concentration dependent weights of the contribution of each permeation mechanism in the channel conductance. Provided the amplitudes g_n of these contributions are quite different, the changes of $p_n(c)$ with the external ion concentration can lead to a rather complex behavior of the channel conductance.

Since the concentration dependences of the throughput and three-ion mechanisms are determined by the same occupancy probability $[p_3(c)]$, it is convenient for further analysis to combine the amplitudes g_3 and g_{tp} into one parameter g_3^{tot} and rewrite (44) in the form

$$g(c) = g_1 p_1(c) + g_2 p_2(c) + g_3^{\text{tot}} p_3(c). \tag{47}$$

Though the parameter g_3^{tot} combines the amplitudes of two different permeation mechanisms, they both involve three-ion interactions in the selectivity filter which is reflected in the identical concentration dependence of their contributions. These two mechanisms together will be referred to as the three-ion mechanisms when we do not need to distinguish between them.

Using the explicit expression (8) for $p_n(c)$ we can write (47) in the form of the ratio of two third degree polynomials in c,

$$g(c) = \frac{g_1 K_1 c + g_2 K_1 K_2 c^2 + g_3^{\text{tot}} K_1 K_2 K_3 c^3}{1 + K_1 c + K_1 K_2 c^2 + K_1 K_2 K_3 c^3}. \tag{48}$$

Equation (48) generalizes the Michaelis-Menten equation (1) (a ratio of first degree polynomials) and our earlier result [18] (a ratio of second degree polynomials) for low-conductance channels. We shall apply (48) to analyze the experimentally known conductance-concentration dependences for different types of potassium ion channels.

The Appendix gives an alternative form for $g(c)$ in terms of ionic effective electrical mobility and average filter occupancy. This form was used in Ref. [18] to analyze the conductance of low-conductance channels assuming that the effective electrical mobility depends only weakly on the external ion concentration and can be approximated by a constant. The Appendix provides an explicit form for the concentration-dependent effective electrical mobility for ions in the selectivity filter of potassium channels.

A. High-conductance channels

As discussed in Ref. [18], high-conductance channels (in contrast to low-conductance channels) have a selectivity filter surrounded by the residues with net negative electrical charge. As an example, there are four negative charges ($-4e$) at a distance of 6–8 Å from the ion permeation pathway surrounding the KcsA selectivity filter [7]. These charges are provided by four carboxyl-carboxylate pairs formed by the side chains of Glu71 and Asp80. The electrostatic interactions of these residues with the permeating cations create a potential energy well in the selectivity filter with a minimum near the S_1 binding site. Other high-conductance channels (e.g., *Shaker*, MthK, BK) whose selectivity filter region structures are believed to be similar to KcsA and whose sequences contain a negatively charged Asp (or sometimes Glu) residue in the position equivalent to Asp80 in KcsA, apparently have similar electrostatic wells along the permeation pathway for cations in the filter. The potential well creates an effective attraction between cations in the filter which is balanced by the ion-ion repulsion. The equilibrium distance between two cations in the well roughly corresponds to the separation between S_1 and S_3 (or S_2 and S_4) binding sites (approximately 7 Å [3,6]).

Analysis of the potassium ion distribution in the selectivity filter of KcsA [27] shows that the filter is predominantly occupied by two ions at physiological symmetrical ion concentrations (and we suggest this is true for other high-conductance channels). This number confirms the proposed earlier interpretation of the potassium ion distribution based on the electron density distribution [6], i.e., that two ions in the filter occupy sites S_1 and S_3 or S_2 and S_4. This also means that two potassium ions in the filter preferentially occupy such binding sites Si as to be at the above described equilib-

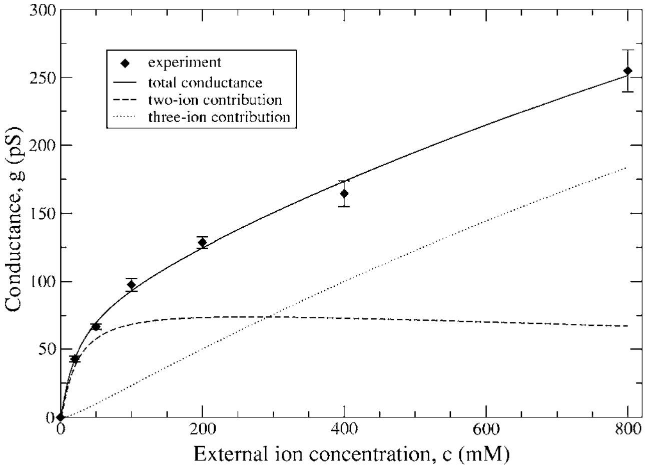

FIG. 2. Conductance-concentration dependence for the KcsA potassium channel and the contributions of different conduction mechanisms. The diamonds show the experimental data points [28]. The solid line is the nonlinear least-squares fit using the polynomial ratio (48). The dashed line is the contribution of the two-ion conduction mechanism and the dotted line is the contribution of the three-ion conduction mechanisms, corresponding to Eq. (47).

rium distance. These two ion configurations allow the third ion to approach the entrance of the filter (from the side with vacant binding site) reaching approximately the same ion-ion separation as the two ions in the filter. (This configuration is favorable for the 'throughput" ion transfer mechanism.)

When an external electrical field is applied, the "polarization" of the predominantly two-ion distribution can play a substantial role in the channel conductance.

We can use (48) to fit the experimentally known concentration dependences of the conductances of high-conductance potassium channels. As an example we consider the recently determined conductance-concentration dependence of the KcsA channel [28]. This dependence and its nonlinear least-squares fit using (47) are shown in Fig. 2. The values of the parameters obtained from the fit are $g_1=5.6$ pS, $g_2=87$ pS, $g_3^{\mathrm{tot}}=893$ pS, $(K_1)^{-1}=3$ mM, $(K_2)^{-1}=23$ mM, and $(K_3)^{-1}=3000$ mM with $\chi^2=140.8$.

It is interesting to note that the amplitudes g_n and g_3^{tot} corresponding to the different conductance mechanisms differ from each other by an order of magnitude. This provides the steady increase of the conductance beyond the initial growth region as the weight is shifted from p_2 to p_3 with the concentration increase. The contributions of the two- and combined three-ion conductance mechanisms as well as the total conductance are shown in Fig. 2.

The parameters K_n allow us to estimate the average occupancy $\bar{n}$ of the KcsA selectivity filter for a given ion concentration. We find there are on average 2.0 ions in the filter at 200 mM external K^+ concentration, which is in a good agreement with the value 2.1 obtained from experiment [27].

Having the values of K_n we can calculate the concentration dependencies of the occupancy probabilities $p_n(c)$ [see Eq. (8) for the KcsA channel for each of the three levels of occupancy $(n=1,2,3)$]. These dependencies are plotted in Fig. 3. It is seen from these plots that the probability to have two ions in the filter (p_2) dominates the other two quantities $(p_1$ and $p_3)$ starting from about 25 mM K^+ and quickly reaches the value 0.8 (close to its maximum 0.85) at about 100 mM K^+, and stays close to this value for the larger c value of this plot. Simultaneously, the probability p_1 drops from approximately 0.5 to 0.2 in the former range (25–100 mM) decreasing below 0.1 after 200 mM. The probability to have three ions in the filter shows an almost linear increase over the whole range of concentration, reaching 0.2 at 800 mM K^+. The behavior of the $p_n(c)$ clearly shows that in the physiological range of ion concentrations the filter remains mostly in two-ion occupancy state.

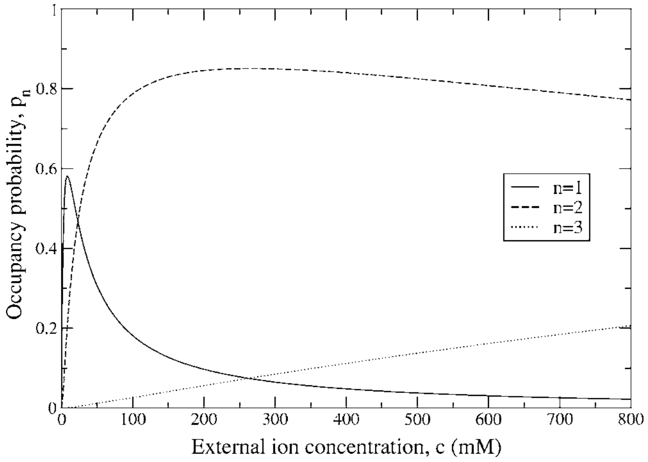

FIG. 3. Concentration dependence of the occupancy probabilities $p_n(c)$ for different occupancy levels $(n=1,2,3)$ of the KcsA selectivity filter.

B. Low-conductance channels

Compared to high-conductance channels, low-conductance channels are characterized by an electrically net neutral nearest environment of the selectivity filter [18]. The repulsion between ions in the filter is not balanced by the interaction with "external" charged residues. Therefore, the two ions in the filter try to occupy the most separated binding sites (i.e., sites S_1 and S_4). This two-ion state (state 23 in Fig. 1) is not favorable for the close approach of a third ion to the filter entrance (which would correspond the 3–4 Å separation with the ion in the filter). The high probability of this state for low-conductance channels substantially decreases the contributions of the "throughput" mechanism and the other three-ion permeation mechanism to the channel conductance.

An analysis of the contribution of the two-ion distribution "polarization" to the conductance of low-conductance channels predicts that this contribution is very small. This is due to the small values of the matrix elements f_{nj} [the differences of the equilibrium fluxes in the left and right directions defined by (21)] which enter (23). In the case of low-conductance channels these values are small because of either small relative probabilities $p_j^{(n)}$ of the states which can be polarized or small differences of the rate constants connected with the symmetrical predominant state 23 (in Fig. 1). This significantly decreases the "polarization" part of A_2, de-

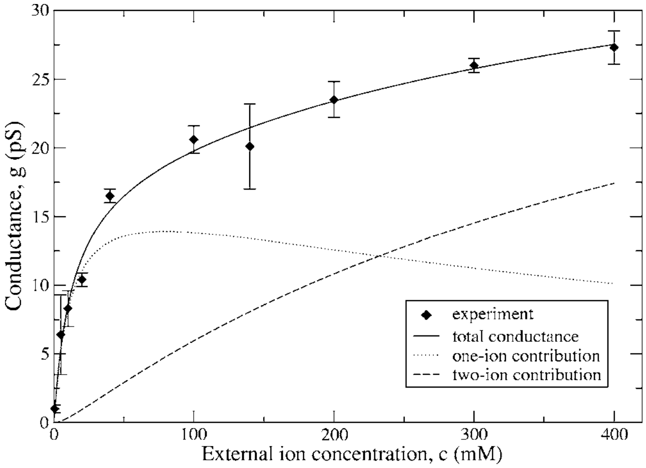

FIG. 4. Conductance-concentration dependence for the Kir2.1 potassium channel and the contributions of one- and two-ion conduction mechanisms. The diamonds show the experimental data points [18]. The solid line is the nonlinear least-squares fit using the polynomial ratio (48). The dotted line is the contribution of the one-ion conduction mechanism and the dashed line is the contribution of the two-ion conduction mechanism, corresponding to Eq. (47).

creasing the amplitude of the $(1 \leftrightarrows 2)$ conductance mechanism (parameter g_2). The increase of the reduced rate constant k_{21} entering g_2 compared to k_{10} in g_1 due to the ion-ion repulsion is also smaller. The smaller repulsion is due to a larger average ion-ion distance for the two-ion distribution the selectivity filter. These two factors can lead to a smaller contribution of the $g_2 p_2$ term in (47) compared with high-conductance channels.

Fitting the conductance-concentration dependence [18] of the Kir2.1 potassium channel by (48) gives us the following values of the parameters: $g_1 = 18.1$ pS, $g_2 = 41$ pS, $(K_1)^{-1} = 12$ mM, $(K_2)^{-1} = 526$ mM, and $K_3 = 0$ with $\chi^2 = 7.45$. The three-ion binding constant K_3 is set equal to zero since in the given experimental range of the external ion concentration (0–400 mM K^+) the possible contributions of the three-ion conduction mechanisms are believed to be very small. For this reason the amplitude g_3^{tot} is left undetermined. The conductance and its nonlinear least-squares fit as a function of c are shown in Fig. 4. Also shown in Fig. 4 are the separate one- and two-ion contributions to the conductance.

Using (4) and the values of the parameters K_1 and K_2 we estimate that the average Kir2.1 filter occupancy is 1.2 ions at 200 mM external K^+ concentration which is much lower than the value 2.0 for the KcsA filter. Essentially, at physiological ion concentrations the Kir2.1 filter is occupied by one ion most of the time. This is in agreement with the suggestion [18] that the shallow electrostatic well along the ion pathway in the Kir2.1 selectivity filter created by the dipoles of Glu-Arg salt bridges in filter region does not fully compensate the ion-ion repulsion.

The values of K_n obtained from the fit allow us to calculate the concentration dependencies of the one- and two-ion occupancy probabilities $p_n(c)$. It is seen from the plots in Fig. 5 that in the physiological range of ion concentrations the filter remains mostly in one-ion occupancy state.

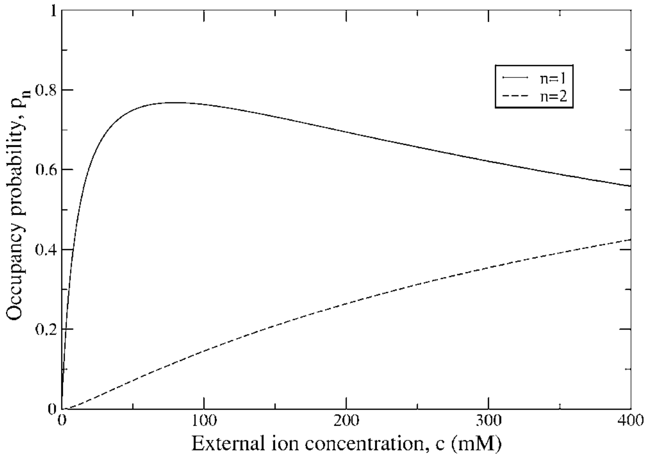

FIG. 5. Concentration dependence of the occupancy probabilities $p_n(c)$ for the two occupancy levels $(n=1,2)$ of the Kir2.1 selectivity filter.

V. DISCUSSION AND CONCLUSIONS

Using the assumption of fast relaxation of the ion distribution inside the selectivity filter compared to the time scale of the ion entry-exit events we have derived an equation for the concentration-dependent ion conductance of potassium ion channels. This equation describes the contributions of different conduction mechanisms to the total channel conductance and is a sum of concentration-independent conductance amplitudes g_n for each permeation mechanism weighted by the equilibrium occupancy probabilities of the filter $p_n(c)$. The changes in $p_n(c)$ with the change of external ion concentration can alter the contributions of the corresponding mechanisms from zero to some maximum values, proportional to g_n [equal to g_n in the case of $p_n(c) \approx 1$]. The behavior of $p_n(c)$, which is determined by the values of the equilibrium binding constants K_n, together with the values of g_n determine the variety of conductance behavior for low- and high-conductance channels. The presence of negatively charged residues around the selectivity filter of the high-conductance channels increases the binding constants causing the larger occupancy probabilities to occur at smaller c compared to the low-conductance channels. Such changes in $p_n(c)$ lead to substantial increases of the contributions of the two- and three-ion permeation mechanisms which have larger conductance amplitudes compared to the one-ion mechanism. All of these result in the higher values of conductance for the high-conductance channels over the wide range of external ion concentrations compared to the low-conductance channels.

As was shown in the preceding sections, the amplitudes of the conductance mechanisms g_n and g_{tp} [see Eqs. (45) and (46)] depend on several different factors. Among them are the equilibrium ion distribution in the selectivity filter described by the state probabilities p_{ni} and the electrical "susceptibility" of this distribution, which largely determine the reduced rate constants $k_{nn'}^{(L,R)}$ and the reduced "polarization" rate constants $k_{nn'}^{(L,R)\ \mathrm{pol}}$, respectively. Both of these factors depend on the free energy surface for the ions in the selectivity filter region which is strongly affected by the protein electrical charge distribution in the vicinity of the selectivity filter.

To elucidate the effects of the different factors on the conductance amplitudes we compare the corresponding values of g_n obtained from the fitting of the conductance-concentration dependencies for KcsA and Kir2.1 as representatives of high- and low-conductance channels. As discussed previously, the amplitude g_n is proportional to two major quantities: the reduced exit rate constant k_{nn-1} and the parameter A_n. To analyze the influence of these two factors on the amplitude g_1 we first compare the values of the one-ion binding constants K_1 for KcsA and Kir2.1. Since K_n can be expressed as the ratio of the entry and exit reduced rate constants [see Eq. (6)], comparing the different K_1's enables us to derive a conclusion about the relative values of these rate constants. Assuming that the entry rate constants k_{01}, i.e., the rate constants for an ion to enter the empty selectivity filter, are approximately the same for KcsA and Kir2.1 (this is a reasonable assumption provided we neglect a possible small difference in the geometry of the filters for the two channels and the small biasing potential of the electrostatic well in the case of KcsA), and taking into account that the value of K_1 for KcsA is four times as large as that for Kir2.1, we conclude that the reduced exit rate constant k_{10} for KcsA is smaller, and is probably the major factor responsible for the lower value of g_1, 5.6 pS vs 18.1 pS for Kir2.1. Thus, the much deeper electrostatic well in the KcsA selectivity filter can be considered as the factor responsible for the reduction of the amplitude for the one-ion permeation mechanism in the case of high-conductance channels.

The opposite situation, however, is observed for the parameter g_2, where its value is twice as large in the case of KcsA than in the case of Kir2.1 (87 pS vs 41 pS). Comparing the values of the two-ion binding constants K_2 (they differ by a factor of 20) one comes to the same conclusion as in the previous case, i.e., that the value of the reduced exit rate constant k_{21} is lower in the case of KcsA. (It is necessary to note, however, that in contrast to entering the empty filter, the entry of the second ion to the preoccupied filter depends on the one-ion distribution in the filter, which can be quite different for these two channels. The ion energy profile for Kir2.1 due to the electrically net neutral nearest environment of the selectivity filter provides a more uniform one-ion distribution, increasing the probabilities for the occupation of the S_1 and S_4 binding sites. When in these sites, an ion prevents the entry of a second ion from the corresponding end of the filter, which can lead to a decrease of the averaged entry rate constant k_{12}. This possible decrease of k_{12} for Kir2.1 contributes to the factor of 20, thus decreasing the actual difference in the reduced exit rate constants k_{21}.) The smaller value of k_{21} for the KcsA channel means that the parameter A_2 is the factor responsible for the larger value of g_2 for KcsA. This parameter includes the "polarization" part which, as was discussed in the Results section, is argued to be much smaller for the Kir2.1 selectivity filter compared to KcsA. The two most probable states of the two-ion distribution in the KcsA filter are expected to be much more "polarizible" than the one most probable state in the Kir2.1 filter.

As discussed earlier in Ref. [18], the fact that the most probable state of the two-ion distribution in the selectivity filter of Kir2.1 is the state where the two ions are in the most distant binding sites S_1 and S_4, drastically decreases the probability of the three-ion and "throughput" permeation mechanisms. We suggest this is true for all low-conductance channels and is the major factor of their much lower conductance at high external ion concentrations taking into account the very large value of the combined amplitude for these two conduction mechanisms g_3^{tot}. In the case of KcsA this value is estimated to be an order of magnitude larger than the value for the two-ion permeation mechanism g_2.

The expression for potassium ion channel conductance derived in this paper enabling the description of both low- and high-conductance channels over a broad range of external ion concentrations is more general compared to the equation obtained earlier in Ref. [18] which was limited to the description of the low-conductance channels. Fitting the available experimental data for the conductances of the KcsA channel allows us to extract the equilibrium binding constants for different ion occupancies of the selectivity filter of this high-conductance channel and to calculate the average number of potassium ions in the filter. The predicted number of 2.0 ions at 200 mM external K$^+$ concentration is in a very good agreement with the experimental value 2.1 in Ref. [27] obtained from the analysis of the electron density of potassium ions in the crystal structure of KcsA in 200 mM K$^+$. The predicted number for Kir2.1 is 1.2 ions at 200 mM K$^+$, and it is hoped that future experiments can test this prediction.

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APPENDIX: EFFECTIVE ELECTRICAL MOBILITY

Using another definition of electrical conductance [29] as the quantity proportional to the average electrical mobility u of charge carriers and the average density of these carriers, the conductance of the selectivity filter can be written in terms of the c-dependent effective electrical mobility u_{eff} of ions in the filter and the average occupancy $\bar{n}$ of the filter as

$$g(c) = \frac{q}{L^2} u_{\text{eff}}(c)\bar{n}(c), \tag{A1}$$

where L is the length of the filter, and $\bar{n}$ is given by (4) and its c-dependence is determined by

$$\bar{n}(c) = \frac{K_1c + 2K_1K_2c^2 + 3K_1K_2K_3c^3}{1 + K_1c + K_1K_2c^2 + K_1K_2K_3c^3}. \tag{A2}$$

Comparing (39) and (A1) and taking into account (A2) we find an expression for the effective electrical mobility,

$$\begin{aligned} u_{\text{eff}}(c) &= \frac{qL^2k_{10}A_1}{2k_BT}\left(p_1 + \frac{k_{21}}{k_{10}}\frac{A_2}{A_1}p_2 + \frac{k_{32}}{k_{10}}\frac{A_3}{A_1}p_3 + \frac{k_{32}}{k_{10}}\frac{A_{tp}}{A_1}p_3\right) \\ &\quad \times (p_1 + 2p_2 + 3p_3)^{-1} \\ &= \frac{g_1L^2}{q}\left(\frac{1 + B_2K_2c + B_3K_2K_3c^2}{1 + 2K_2c + 3K_2K_3c^2}\right), \end{aligned} \tag{A3}$$

where $B_2 = g_2/g_1$ and $B_3 = g_3^{\text{tot}}/g_1$.

Equations (A1) and (A3) generalize the result in Ref. [18] for the concentration dependence of the conductance of low-conductance potassium channels, where u_{eff} was assumed to be independent of c. Since for low-conductance channels, as was discussed above, $p_3(c)$ is negligible in the physiological concentration range ($K_3 c \ll 1$), and the ion distribution is strongly unfavorable for the "throughput" ion transfer conduction mechanism (very small probabilities $p_t^{(R,L)}$ and, accordingly, small values of the parameter g_{tp}), we can neglect the B_3 term in (A3). In this case, u_{eff} becomes independent of c when B_2 is close to 2, i.e.,

$$B_2 = \frac{g_2}{g_1} = \frac{k_{21} A_2}{k_{10} A_1} \approx 2. \tag{A4}$$

B_2 is determined by the ratio of the reduced exit rate constants and the ratio of the parameters A_2 and A_1. Due to the ion-ion repulsion k_{21} is always larger than k_{10}. But for low-conductance channels, where the predominant state in the two-ion distribution is the (23) state, the increase in reduced exit rate constant is not as large as for high-conductance channels, where two ions in the filter are closer to each other with the predominant states being (22) and (25). Also, due to the symmetry of the (23) state, the ion distribution "polarization" effects (which contribute to parameters A_2) are assumed to be considerably smaller for the low-conductance channels. These two factors are responsible for the lower value of B_2 in the case of the low-conductance channels and the small or negligible c dependence of u_{eff}. Using the values of the parameters g_1 and g_2 for Kir2.1 we estimate $B_2=2.3$, which is sufficiently close to 2, the value for which u_{eff} is independent of c [see (A3) and recall thatK_3 is negligible for low-conductance channels].

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