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# Non-Michaelis-Menten kinetics model for conductance of low-conductance potassium ion channels

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A reduced kinetics model is proposed for ion permeation in low-conductance potassium ion channels with zero net electrical charge in the selectivity filter region. The selectivity filter is assumed to be the only conductance-determining part of the channel. Ion entry and exit rate constants depend on the occupancy of the filter due to ion-ion interactions. The corresponding rates are assumed slow relative to the rates of ion motion between binding sites inside the filter, allowing a reduction of the kinetics model of the filter by averaging the entry and exit rate constants over the states with a particular occupancy number. The reduced kinetics model for low-conductance channels is described by only three states and two sets of effective rate constants characterizing transitions between these states. An explicit expression for the channel conductance as a function of symmetrical external ion concentration is derived under the assumption that the average electrical mobility of ions in the selectivity filter region in a limited range of ion concentrations does not depend on these concentrations. The simplified conductance model is shown to provide a good description of the experimentally observed conductance-concentration curve for the low-conductance potassium channel Kir2.1, and also predicts the mean occupancy of the selectivity filter of this channel. We find that at physiological external ion concentrations this occupancy is much lower than the value of two ions observed for one of the high-conductance potassium channels, KcsA.

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## I. INTRODUCTION

Ion channels are integral membrane proteins that provide pathways for relatively fast ion movement across cell membranes. They regulate ion passage by "opening" or "closing" a pathway at a site called the "gate," stochastically or in response to some external factor such as a change of membrane voltage, mechanical tension, ligand binding, etc. Their ability to conduct ions can be selective with respect to ion charge and size.

Ion channel conductance, selectivity, and gating have been studied experimentally and theoretically for a long time (for reviews, see [1,2]). The conductive pathways of ion channels are water-filled pores that span the channel proteins, allowing ions to pass from one side of a membrane to the other along an electrochemical gradient. In the pore, ions continue to have strong interactions with pore water molecules maintaining (fully or partially) their hydration shells. The narrowest part of the pore in an "open" selective channel serves as a selectivity filter. In this part of the channel the ions, being partially or fully dehydrated, make direct contacts with protein groups. The strong and specific interactions with these groups replace the interaction with the hydration shell water molecules [3,4]. These interactions, as well as the interactions with the more distant charged groups in the selectivity filter environment, determine the selectivity and conductance properties of the channels [5].

The conductances of the various selective channels, particularly potassium channels, differ from each other by more than an order of magnitude, ranging from 10 to 40 pS for low-conductance potassium channels [e.g., for inward rectifier $K^+$ channels (Kir)] to 100–300 pS for high-conductance potassium channels (e.g., KcsA, MthK, BK). The most conductive potassium channels (BK) can pass ions at a remarkable rate, $\sim 10^8$ ions per second [6–8]. This very large range of potassium channel conductances is one of the issues that are currently unresolved. Much recent experimental effort is devoted to illuminating this issue [8,9]. In this paper we present a model to account for the conductance of the low-conductance potassium channels and discuss a difference in conduction mechanisms of the low- and high-conductance channels.

There have been several theoretical attempts to develop simple kinetics models for the concentration-dependent ion permeation kinetics in single-file multi-ion channels. One of the simplest equations, initially developed for ion channels with single-ion occupancy of the pore [10], but frequently used empirically for multi-ion channels [1], is the Michaelis-Menten equation

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

where $g_{max}$ is the maximum (saturation) conductance of the channel and $c_{1/2}$ is the ion concentration at which $g = g_{max}/2$. This equation is also widely used in enzyme kinetics, where the constant $K_M \equiv c_{1/2}^{-1}$ is referred to as the binding constant in the equilibrium theory, or as the Michaelis constant in the steady-state theory.

To account for multiple-ion effects, including possible inhibition of conductance at high levels of external ion concentration $c$, a single-vacancy model has been proposed [11,12]. In this model, the pore can be occupied by either $n$ or $n-1$ ions where $n$ is equal to the number of ion binding sites $N$ in the pore (selectivity filter). At very high $c$, the decrease of internal ion transition rates due to depopulation of the $(n-1)$-occupied states with an ion vacancy leads to a decrease of channel conductance. Mathematically, the denominator of Eq. (1) acquires a $c^2$ term which is proportional to the reciprocal rate constant for the ion transitions inside the channel [12].

Similar to the single-vacancy model, a two-state occupancy model ($n$ and $n-1$ ions in the selectivity filter) has been proposed to describe the multi-ion permeation kinetics in KcsA or other structurally equivalent channels [13,14]. In this model, $n$ is not necessarily equal to $N$, and ion translocation inside the filter is assumed to be fast, compared to the ion exit or entry rates. The latter assumption and the assumption that the filter always contains at least $n-1$ ions lead to an effective single-ion kinetics which is described by the Michaelis-Menten equation.

The purpose of this work is to propose a theory for the conductance of the low-conductance potassium channels based on a simplified kinetics model of the selectivity filter. This theory, in contrast to the earlier kinetics models, takes into account multiple-occupancy states of the selectivity filter of multi-ion channel, and is able to describe the permeation kinetics over a broad range of external ion concentration, where the possible change in $n$ is greater than 1. Accordingly, the model takes into account the changes of exit and entry rate constants which arise from different degrees of ion-ion interaction for different numbers of ions inside the selectivity filter of the channel. These changes can manifest themselves as a deviation of multi-ion kinetics from the Michaelis-Menten behavior. The theory and the underlying kinetics model are based on several simplifying assumptions. These assumptions are valid for the low-conductance potassium channels with zero net electrical charge in the selectivity filter region (e.g., for the KirBac2.1 channel and for the members of the Kir family: Kir2.1, Kir3.1, Kir3.4, Kir4.1, Kir4.2, Kir6.1) but are probably invalid for high-conductance channels, in particular for the well studied KcsA channel. The theory is shown to provide a good representation of the channel conductance $g$ as a function of the ion concentration $c$ in the external bathing solutions when tested against data for the Kir2.1 channel. The mean occupancy of the selectivity filter as a function of $c$ is also predicted.

## II. THEORY

The recent determinations of the crystal structures of the bacterial potassium channels KcsA [15], MthK [16], KvAP [17], and KirBac1.1 [18] have provided a template for the pore structure of most potassium channels. In accordance with these structures the current view of a typical potassium channel is that the conductive pore of the "open" channel consists of two major parts: a water-filled cavity wide open to the cytoplasmic (intracellular) region, and a rather narrow (in diameter) selectivity filter connecting the pore to the extracellular region. Since ions in the water cavity are fully hydrated and their diffusive motion from or to the intracellular region is relatively unrestricted, we assume the conductance of the wide open water cavity, $g_C$, to be high compared to the conductance of the selectivity filter, $g_F$. These conductances contribute to the total channel conductance $g$ in the following way:

$$g^{-1} = g_C^{-1} + g_F^{-1}, \tag{2}$$

i.e., the resistances in series add to give the total. Because of the above assumption ($g_C \gg g_F$), $g_C$ contributes a negligible amount to the total channel conductance, i.e., we have

$$g \approx g_F. \tag{3}$$

In the following, we omit the index $F$ on $g$ and consider the conductance of the selectivity filter only.

A general equation that describes the conductance of a selective ion-conducting pore of length $L$ and average cross-sectional area $S$ connecting two bathing ion solutions (reservoirs) with symmetric (equal) ion concentrations and containing water and only one type of permeating ion carrying charge $q$ can be written [19], using the definitions of $g$ and electrical mobility $u$, as

$$g = qu_{eff}c_{ch}S/L, \tag{4}$$

where $u_{eff}$ is the effective (average) electrical mobility of the ions in the pore region, including the contributions of the entry and exit of ions to and from the pore, and $c_{ch}$ the average concentration of ions inside the pore.

Assuming the geometry of the selectivity filter (parameters $S$ and $L$) does not significantly change with a change of the external ion concentration $c$, the only $c$-dependent factors that affect the channel conductance are $u_{eff}$ and $c_{ch}$. This assumption is valid unless conformational changes of the protein in the selectivity filter region occur which drastically distort the filter geometry with a change of an external ion concentration. In addition, in the following we assume that the change of ion concentration does not produce any other changes in the filter geometry that can affect $u_{eff}$ through a change of ion-protein interactions. This last assumption may not be valid for some potassium channels, as was recently discovered in the cases of the KcsA channel [20] and the Kir1.1 (ROMK) channel [21]. In the latter work it was shown that inactivation of Kir1.1 due to membrane depolarization is dependent on ion concentration, but not when extracellular loop residues of the channel are replaced by Kir2.1 homologous residues.

The quantity $c_{ch}$ can be expressed in terms of the average number of ions in the selectivity filter $\bar{n}(c)$ (which is also called the average occupancy number or the average population of the selectivity filter) as $c_{ch}=\bar{n}/SL$. Thus, Eq. (4) can be rewritten as

$$g = q u_{eff}\bar{n}/L^2. \tag{5}$$

To find the concentration dependence of $u_{eff}(c)$ and $\bar{n}(c)$ we need to analyze a kinetics model of the channel, which determines a set of differential equations for the probabilities of the channel to be in different states. These states describe possible ion distributions over the ion binding sites in the selectivity filter of the channel. Transitions between the states correspond to the ion motion in the pore. The values of $u_{eff}(c)$ and $\bar{n}(c)$ are directly connected to the changes of the state probabilities and the transitions rates with external ion concentration.

In accordance with the known crystal structures of $K^+$ channels [15–17] the selectivity filter of a typical potassium channel contains four $K^+$ ion binding sites. In these sites dehydrated potassium ions are coordinated by the backbone carbonyl oxygen atoms that compensate for the missing interactions with the water oxygen atoms of the first hydration shell of the ion. The refined structure of KcsA [3,4] and theoretical estimations [24] show that the KcsA selectivity filter is surrounded by four negative charges provided by carboxyl-carboxylate pairs formed by the side chains of Glu71 and Asp80. These charges near the ion pathway create a rather deep electrostatic well in the selectivity filter [25], attractive for cations. Similar wells apparently also exist in MthK, BK, and other high-conductance channels whose sequences contain a negatively charged Asp residue in the selectivity filter position equivalent to Asp80 in KcsA. In contrast to the latter cases, the selectivity filters of low-conductance channels (Kir1.1, Kir2.1, Kir3.1, Kir3.4, Kir4.1, Kir4.2, Kir6.1, sWIRK) contain in the positions equivalent to positions 71 and 81 of KcsA both negatively (Glu) and positively (Arg) charged residues which form salt bridges with zero net electrical charge [26–28,5]. The dipole moments of these salt bridges cannot create as deep an electrostatic well along the ion pathway as the uncompensated negative charges in KcsA or other high-conductance channels. X-ray data analysis [3,20] and molecular (MD) and Brownian dynamics simulations [22,23,29] indicate that the KcsA filter under physiological (50–200 mM) or even higher external ion concentrations is occupied mostly by two potassium ions. It then follows that the probability of finding three or four ions in the selectivity filter of the low-conductance channels is very small for ion concentrations not far from physiological, due to strong repulsions among three or more ions, which are not compensated by the interactions with external negative charges as in the case of KcsA or other high-conductance channels (Kv, MthK, BK). This allows us to neglect the corresponding three- and four-ion occupancy states in the kinetics model for low-conductance channels. (For details see Appendix A.) For the same reason (i.e., charge distribution difference in the selectivity filter region), we also neglect possible three-ion transient states, occurring when the third ion reaches the top of the barrier near either end of the filter, followed by concerted motion of all three ions through the filter [3]. This type of ion motion is considered a major conduction mechanism in KcsA by MacKinnon and co-workers [3,20]. The above argument suggests that the rates of the transitions between the appropriate two-ion states and the three-ion transient states are negligibly small for low-conductance potassium channels, for not too high external ion concentrations (for details see Appendix B). The charge distribution (and hence kinetics) differences between the Kir channels and KcsA just discussed are responsible for the fact that the conductance is an order of magnitude smaller for the Kir family of channels than for KcsA, MthK, or BK.

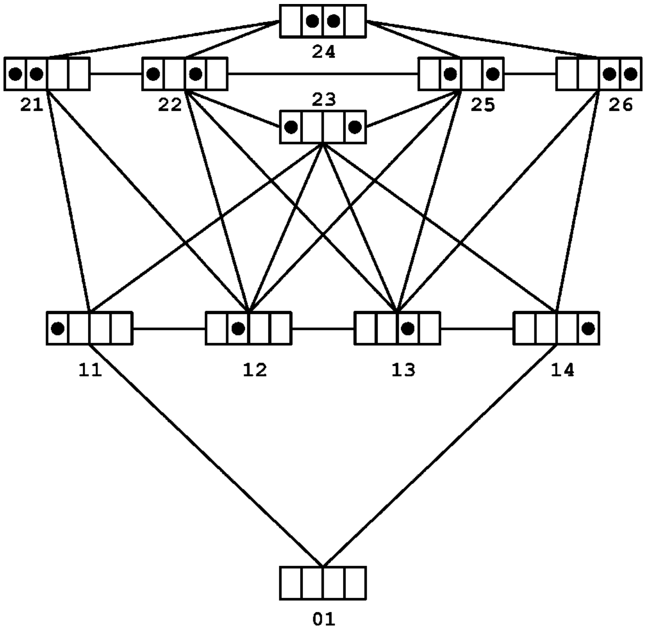

FIG. 1. Kinetics model for the potassium channel selectivity filter.

With the above restriction, we are led to a kinetics scheme of the selectivity filter depicted in Fig. 1. The proposed scheme consists of 11 states of the filter corresponding to different binding site occupations; six states with double occupancy, four states occupied by a single ion, and one empty state. The lines connecting states indicate possible transitions between two states that are characterized by two rate constants (forward and backward). Possible transitions include the concerted (i.e., cooperative) transitions, when two ions cross barriers separating binding sites, described by one transition in the kinetics scheme [30]. In labeling the states $ni$, and corresponding probabilities $p_{ni}$, the first index $n=0$, 1, 2 corresponds to the state occupancy number and the second index denotes the substate within the same occupancy. A transition that changes the occupancy number $(ni \rightarrow n'j, n \neq n')$ corresponds to the entry $(n<n')$ or the exit $(n>n')$ of an ion from the filter. The corresponding rate constants $k_{ni,n'j}$ are called entry or exit rate constants. When we need to distinguish between the entry or exit from the left $(L)$ or right $(R)$ sides of the filter we will use a superscript $(L)$ or $(R)$ in the notation for the rate constant (e.g., $k^{(L)}_{ni,n'j}$). Transitions between the states with the same occupancy number $(ni$

$\rightarrow nj$) do not involve entry or exit of an ion to or from the selectivity filter. The corresponding rate constants are denoted as $k_{ni,nj}$.

It is clear that even this restricted kinetics scheme with about 50 parameters (rate constants) and the corresponding set of differential equations for the probabilities $p_{ni}$ are rather complicated for a complete analytical analysis. There has been some previous analytical work [31–33] restricted to symmetrical two-ion channels or to simplifying limits, but $g$ was not derived as an explicit function of $c$.

Several approaches for numerical analysis of kinetics schemes have been also proposed [34,35,1]. In these approaches the rate constants for transitions between singly and multiply occupied states are obtained from the single-ion free energy profile. The kinetics schemes proposed in these studies do not include possible concerted ion transitions, the rate constants for which are difficult to estimate.

Experimental data [3] and MD simulation results [36] suggest that at equilibrium the ion motion inside the selectivity filter (i.e., transitions between states with the same occupancy number, $ni \rightarrow nj$) occurs on a much faster time scale than the transitions that change the occupancy number, $ni \rightarrow n'j$. Here, the time scale is determined by the reciprocal rate of the unidirectional equilibrium ion flux for the transitions between states. This implies that the relaxation time for the equilibration between states with the same occupancy number is much smaller than the average time between entry or exit events at any external ion concentration. It also implies that under the change of the external ion concentration $c$, at equilibrium, the relative probabilities of the states with the same occupancy number $n$ are unchanged. We also assume that for very small deviations from equilibrium due to a small voltage difference ($q\Delta V \ll k_B T$) across the membrane (i.e., very small steady-state currents through the channel) these relative probabilities are virtually unchanged. Here $k_B$ is Boltzmann's constant and $T$ the temperature. We can express these relative probabilities as the conditional probabilities $p_i^{(n)}$ [usually denoted as $p(i|n)$] to find the selectivity filter in the substate $i$, given the prescribed value of the occupancy number $n$. According to the standard rule of probability theory, we have

$$p_i^{(n)} = p_{ni}/p_n, \tag{6}$$

where

$$p_n = \sum_j p_{nj} \tag{7}$$

is the probability to have $n$ ions in the selectivity filter irrespective of substate $j$.

The quantities $p_n$ (as well as $p_{ni}$) change with $c$ and are the only quantities that determine the average occupancy number $\bar{n}$. The assumption in the last paragraph ($p_i^{(n)} \simeq \text{const}$) allows us to ignore the details of the ion motion inside the selectivity filter for the determination of $\bar{n}$ or $c_{ch}$. Thus, we can describe our system as a set of "generalized" states $s_n$ characterized by the occupancy numbers $n$ and the probabilities $p_n$. Transitions between these new states correspond to the entry or exit of an ion from the selectivity filter and are governed by the reduced effective rate constants $k_{n,n'}^{(L)}$, and $k_{n,n'}^{(R)}$. With $p_i^{(n)}$ unchanged, these quantities are constants (independent of $c$) and can be obtained as a result of an averaging of the entry or exit rate constants $k_{ni,n'j}$ over all possible initial states with the occupancy number $n$ and summing over the final states with the occupancy number $n'$, i.e.,

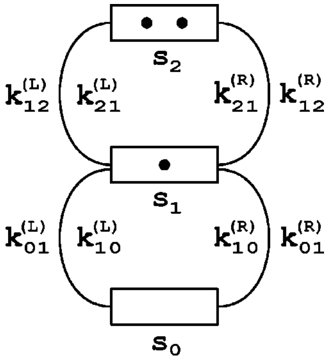

FIG. 2. Simplified effective kinetics model for the selectivity filter.

$$k_{n,n'}^{(L)} = \sum_{ij}{}' p_i^{(n)} k_{ni,n'j}^{(L)}. \tag{8}$$

Here, $\Sigma'_{ij}$ denotes a sum over those pairs of states that correspond to the ion entry (or exit) from (or to) the $L$ bathing solution; a similar expression holds for $k_{n,n'}^{(R)}$.

These new states and the corresponding transitions provide the reduced kinetics scheme that is presented in Fig. 2. Each transition increases or decreases the occupancy number and corresponds to an ion entry or exit event. Comparing this with the scheme of Fig. 1, we see that the number of states of the selectivity filter is reduced from 11 to 3 and the number of rate constants from 52 to 8.

The kinetics model corresponding to the scheme in Fig. 2 can be described by the rate equations

$$\dot{p}_1 = -p_1(k_{10} + k_{12}c) + p_2 k_{21} + p_0 k_{01} c,$$

$$\dot{p}_2 = -p_2 k_{21} + p_1 k_{12} c, \tag{9}$$

together with the normalization equation

$$p_0 + p_1 + p_2 = 1, \tag{10}$$

where $\dot{p}_n = dp_n/dt$, $t$ is the time, and, for brevity, $k_{nn'} \equiv k_{n,n'}^{(L)} + k_{n,n'}^{(R)}$.

At equilibrium or in a steady-state regime we have $\dot{p}_n = 0$. Defining equilibrium constants $K_1$ and $K_2$, where

$$K_1 = k_{01}/k_{10}, \quad K_2 = k_{12}/k_{21}, \tag{11}$$

we can write the equilibrium or steady-state solution of Eqs. (9) and (10) as

$$p_0(c) = \frac{1}{1 + K_1 c + K_1 K_2 c^2}, \tag{12}$$

$$p_1(c) = \frac{K_1c}{1 + K_1c + K_1K_2c^2}, \tag{13}$$

$$p_2(c) = \frac{K_1K_2c^2}{1 + K_1c + K_1K_2c^2}. \tag{14}$$

It is easy to see that $p_1 = p_0K_1c$ and $p_2 = p_1K_2c$, and that $p_n = p_{n-1}$ at $K_nc = 1$. Note that $K_1$ and $K_2$ are the ratios of the sums of entry and exit reduced effective rate constants (11). We expect that due to electrostatic repulsion in the filter region, with the increase of $n$, exit constants $k_{nn-1}$ become larger and entry constants $k_{nn+1}$ become smaller. This leads to the inequality $K_2 < K_1$.

The behavior of $p_n$ is determined by the ratio of $K_1$ and $K_2$. If $K_2 \ll K_1$, in the range of $c$ where $K_2c \ll 1$, the behavior of $p_1(c)$ is close to that of Michaelis-Menten kinetics. In this range of $c$ we also have $p_2(c) \sim c^2$ when $K_1c \ll 1$ and $p_2(c) \sim c$ when $K_1c \gg 1$.

Equations (12)–(14) allow us to evaluate the equilibrium or steady-state average occupancy number $\bar{n}(c)$,

$$\bar{n}(c) = \sum_{n=0}^{2} np_n = \frac{K_1c + 2K_1K_2c^2}{1 + K_1c + K_1K_2c^2}. \tag{15}$$

We see that in the reduced kinetics model the description of the concentration dependence of $\bar{n}$ involves only two parameters $K_1$ and $K_2$, in contrast to the 48 parameters for the full scheme in Fig. 1.

Using Eq. (15) we rewrite the concentration dependence of the channel conductance (5) as

$$g(c) = \frac{q}{L^2}u_{eff}\frac{K_1c + 2K_1K_2c^2}{1 + K_1c + K_1K_2c^2}. \tag{16}$$

The behavior of the effective electrical mobility $u_{eff}(c)$ is the only quantity remaining to determine. We give a qualitative argument which suggests that for a restricted range of external ion concentrations (such that the probability $p_3$ of the states with three ions is negligibly small) $u_{eff}$ is independent of $c$ in first approximation. According to its definition, the electrical mobility of a charged particle determines its drift velocity due to an applied electric field, and is inversely proportional to the friction on the particle, or to the rate of its kinetic energy dissipation. In homogeneous systems (e.g., ions in solution), it is known that the friction depends on the average environment of an ion and depends only very weakly on ion concentration; it may be considered in the first approximation as a constant. Thus it seems reasonable to assume that here also $u_{eff}$ depends relatively weakly on ion concentration, and hence in the first approximation to consider that $u_{eff}$ does not depend on the external ion concentration. We thus take $u_{eff}$ in Eq. (16) a constant.

This allows us to rewrite Eq. (16) as

$$g(c) = g_0\frac{K_1c + 2K_1K_2c^2}{1 + K_1c + K_1K_2c^2} = g_0\bar{n}(c), \tag{17}$$

where $g_0 = qu_{eff}/L^2$ is the third parameter in the conductance description (in addition to $K_1$ and $K_2$). The physical meaning of $g_0$ is evident from Eq. (17); it is the channel conductance for the average filter occupancy of unity ($\bar{n} = 1$). Equation (17) has been derived for a restricted range of external ion concentrations such that there are no more than two ions in the selectivity filter at any given instant. This limits the validity of Eq. (17) to the $c$ range of $0 \leqslant c \lesssim K_2^{-1}$. At higher $c$, the neglect of three- and four-ion occupancy states and the assumption of constant $u_{eff}$ may not be valid. With the average occupancy number approaching the number of ion binding sites in the selectivity filter, $u_{eff}$ will eventually decrease, leading to a decrease of $g$ at very large $c$; see [1,34] for a general numerical study, [12] for a theoretical analysis of the “single-vacancy” model of ion permeation, and [37] for an experimental observation for Kir1.1 (ROMK1).

## III. RESULTS AND DISCUSSION

Equation (17) describes the concentration-dependent conductance of a selective potassium channel in which the selectivity filter provides the main contribution to the resistance of the channel and is surrounded by residues with zero net electrical charge. It was derived for a very small voltage difference $\Delta V$ across the membrane, but, since $g$ is a constant in Ohm’s law, Eq. (17) will be valid in the full linear response range of $\Delta V$, where $i \propto \Delta V$. The equation for $g(c)$ is limited to the range of external potassium ion concentrations for which the selectivity filter is occupied by no more than two ions. We show in Appendix A that adding to the kinetics scheme in Fig. 1 the states with three and four ions in the selectivity filter modifies the equation for $\bar{n}(c)$, but leaves the conductance near the physiological range of $c$ virtually unaffected.

As is shown in Appendix B, electrical neutrality of the nearest environment of the selectivity filter precludes the so-called throughput cycle conduction mechanism in this type of channel at these relatively low ion concentrations. The absence of this mechanism, which if present would drastically increase the effective electrical mobility, allows us to consider $u_{eff}$ as a concentration-independent parameter of the conductance model.

It was previously noted that due to repulsion between potassium ions the constants $K_1$ and $K_2$ are such that $K_1 > K_2$ (or even $K_1 \gg K_2$). Under such conditions the behavior of $g(c)$ at low concentrations ($K_1c < 1$) is linear with $c$ with the slope $g_0K_1$. At higher concentrations ($K_1c \gg 1$ but $K_2c < 1$) following $p_1$ attaining its maximum, the behavior of the conductance is determined mostly by $p_2$. At these concentrations the conductance rises almost linearly with $c$ as $g(c) \simeq g_0[1 + p_2(c)]$ with the slope $g_0K_2$. The crossing point of the extension of this second quasilinear segment with the $g$ axis gives us an approximate value of $g_0$.

We can use Eq. (17) to fit the experimentally known concentration dependences of the conductances of potassium channels with net neutral nearest environment of the selectivity filter. We give an example for illustration. The recently determined conductance-concentration dependence of the Kir2.1 potassium channel [38] and its nonlinear least-squares fit using Eq. (17) are shown in Fig. 3. The values of the parameters obtained from the fit are $g_0 = 18.3$ pS, $K_1^{-1} = 12.6$ mM, and $K_2^{-1} = 397$ mM, with $\chi^2 = 7.74$. For compari-

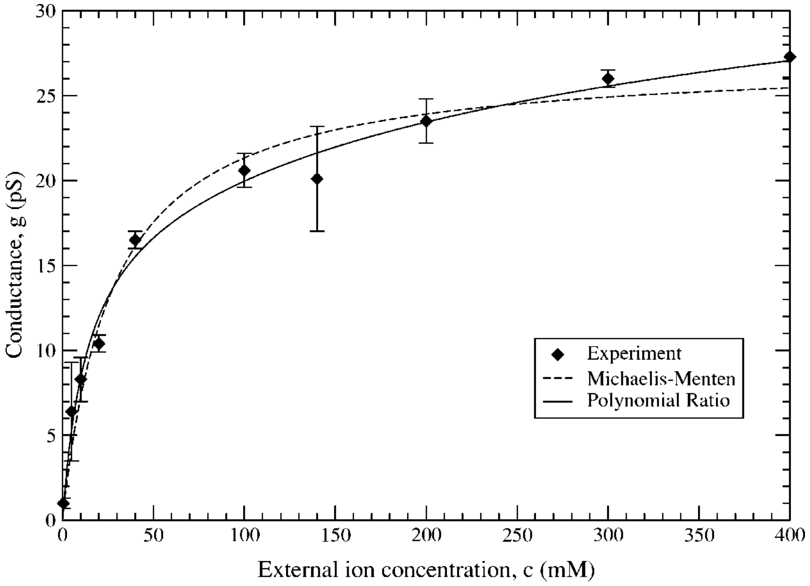

FIG. 3. Conductance-concentration dependence for the Kir2.1 potassium channel. The diamonds show experimental data points [38]. The solid line is the nonlinear least-squares fit using the polynomial ratio (17). The dashed line is the nonlinear least-squares fit using the Michaelis-Menten equation (1).

son, the plot of the nonlinear least-squares fit of the Michaelis-Menten equation (1) is also presented in Fig. 3. The values of the parameters in Eq. (1) that produce the best fit of the experimental data are $g_{max}=27.2$ pS and $c_{1/2}=27.5$ mM, with $\chi^2=19.8$.

Figure 3 clearly shows the difference between Eqs. (17) and (1). The curve (17) has a steeper slope at small $c$ (0–10 mM) and fits better the experimental points in this range of ion concentration than the curve (1). At higher concentrations (100–400 mM), the slope of (17) is again steeper than that of (1) and the curve (17) at 400 mM is far from saturation, in contrast to the Michaelis-Menten curve (1) which is already close to saturation. The much smaller value of $\chi^2$ for the fit by Eq. (17) implies an overall better fit than by using the curve (1). The facts that our model fits the data for the low-conductance Kir2.1 channel and that physically reasonable values for the parameters are obtained $(K_2/K_1 \approx 1/30)$ lend credence to the model.

Using Eq. (15) and the values of the parameters $K_1$ and $K_2$ we estimate that there are on average 1.3 ions in the selectivity filter of Kir2.1 at 200 mM external $K^+$ concentration and 1.5 ions at 400 mM external $K^+$ concentration (the highest concentration used in [38]). These values are quite different from the values estimated for the KcsA channel: 2 ions in the selectivity filter when the external $K^+$ concentration is higher than about 20 mM [3] and 2.1 ions at 200 mM [20]. Such a difference is expected and easily explained by the different electrical charge distributions in the selectivity filter region (i.e., outside the immediate pore-lining region of the selectivity filter). As was noted, Kir2.1 is a potassium channel with zero net electrical charge in the selectivity filter region. For this type of channel, a deep electrostatic well due to negatively charged groups in the vicinity of the selectivity filter, as in the case of KcsA, does not exit. Accordingly, the equilibrium binding constants $K_n$ are much smaller, and $\bar{n}(c)$ is shifted toward higher external ion concentrations. In addition, the electrostatic repulsion between two ions in the selectivity filter in such a shallow well leads to an ion distribution with the two ions tending to opposite binding sites of the filter. This type of two-ion distribution is not favorable for the throughput cycle conduction mechanism (see Appendix B) as the third ion approaching the selectivity filter experiences stronger repulsion.

An Eadie-Hofstee (EH) plot ($g$ vs $g/c$) of the same Kir2.1 conductance data is given in Fig. 4. The curves corresponding to Eqs. (17) and (1) are also presented. It is clearly seen that the behavior of an EH plot of the transformed polynomial ratio (17) corresponds at least qualitatively to the experimental data, showing a change of the slope in the EH plot, whereas the transformed Michaelis-Menten curve (1) is represented by a straight line and shows more dramatically the deviations from the experimental data. The majority of the data points lie along two straight lines and cannot be represented by one transformed Michaelis-Menten curve in both the small- and large-$c$ ranges of ion concentrations simultaneously. A similar situation arises for the high-conductance channel KcsA, where the use of two sets of parameters for the description of channel conductance by Michaelis-Menten equation is proposed [14]. Separately, they describe the low- and high-$c$ behaviors of the conductance, but are not combined in one kinetics model. Our approach allows the appropriate description of the conductance in the small- and large-$c$ ranges in one model, and has one less parameter than two separate Michaelis-Menten models. To be fair, Nelson [14] applies his method to a high-conductance channel, which our model cannot handle.

The parameter $g_0$ is defined as the conductance at $\bar{n}=1$, where the contribution of two-ion interactions in the selectivity filter is small. We can expect that the equivalent quantity for the high-conductance potassium channels (e.g., KcsA) is smaller, due to the deeper electrostatic well. However, the divergence of the $g_0$ values among the low-conductance channels may be larger than the difference between low- and high-conductance channels.

We conclude that the reduced kinetics model proposed in this paper provides an improved description of the conductance of a specific type of potassium channel (i.e., the low-conductance type) by taking into account the change in ion entry and exit rate constants due to an increase of ion-ion

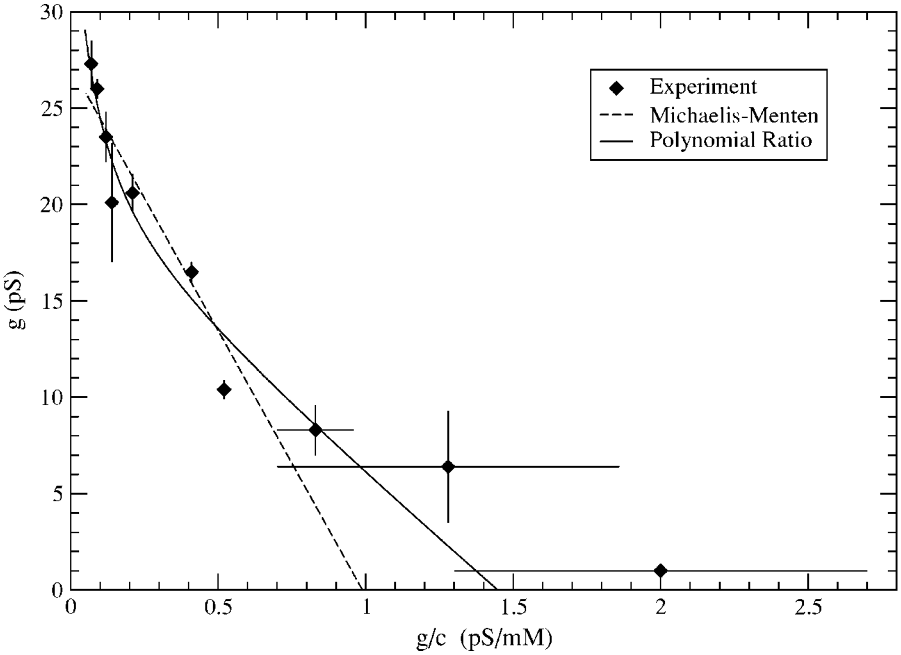

FIG. 4. The Eadie-Hofstee plot of the conductance of the Kir2.1 channel. The diamonds show experimental data points [38]. The solid line is an EH plot of the transformed polynomial ratio (17) shown in Fig. 3. The dashed line is an EH plot of the transformed Michaelis-Menten equation (1) shown in Fig. 3.

interactions as more ions enter in the selectivity filter of the channel. In this paper we applied the theory to Kir2.1 since extensive data $g$ vs $c$ have been determined expressly for this purpose. When such data become available for other low-conductance potassium channels (Kir3.1, etc.) our theory can be tested for them. Work is in progress to extend the model to cases where the current theory does not apply, i.e., to the high-conductance potassium channels with net negative electrical charge surrounding the selectivity filter, e.g., KcsA, MthK and BK. The latter requires further development of the model to include the concentration dependence of the effective electrical mobility $u_{eff}$.

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## APPENDIX A: EXTENSION TO MORE THAN TWO IONS IN THE FILTER

The kinetics scheme in Fig. 1 is restricted to the states where there are no more than two ions in the selectivity filter. This is a reasonable assumption for not too high external ion concentrations. For arbitrarily large concentrations the probabilities to have three or even four ions in the selectivity filter may not be negligible. Adding to the kinetics scheme in Fig. 1 the states with three ions (four possible states) and four ions (one state) will introduce two additional generalized states $s_3$ and $s_4$ to the reduced kinetics scheme in Fig. 2. With these two new states and corresponding new reduced effective rate constants ($k_{nn'}$), the rate equations (9) change to the following:

$$\dot{p}_n = - p_n(k_{nn-1} + k_{nn+1}c) + p_{n+1}k_{n+1n} + p_{n-1}k_{n-1n}c. \tag{A1}$$

In Eq. (A1), the index $n=0,1,\ldots 4$, and the values of $p_{n'}$ and $k_{n'n}$ (or $k_{nn'}$) are equal to 0 when index $n'=n-1<0$ or $n'=n+1>4$. The normalization equation (10) is now

$$\sum_{n=0}^{4} p_n = 1. \tag{A2}$$

Introducing

$$K_3 = k_{23}/k_{32}, \quad K_4 = k_{34}/k_{43}, \tag{A3}$$

in addition to the already defined $K_1$ and $K_2$, we find equilibrium or steady-state solutions of Eq. (A1) as before,

$$p_0(c) = \frac{1}{1 + K_1c + K_1K_2c^2 + K_1K_2K_3c^3 + K_1K_2K_3K_4c^4}, \tag{A4}$$

$$p_1(c) = \frac{K_1c}{1 + K_1c + K_1K_2c^2 + K_1K_2K_3c^3 + K_1K_2K_3K_4c^4}, \tag{A5}$$

$$p_2(c) = \frac{K_1K_2c^2}{1 + K_1c + K_1K_2c^2 + K_1K_2K_3c^3 + K_1K_2K_3K_4c^4}, \tag{A6}$$

$$p_3(c) = \frac{K_1K_2K_3c^3}{1 + K_1c + K_1K_2c^2 + K_1K_2K_3c^3 + K_1K_2K_3K_4c^4}, \tag{A7}$$

$$p_4(c) = \frac{K_1K_2K_3K_4c^4}{1 + K_1c + K_1K_2c^2 + K_1K_2K_3c^3 + K_1K_2K_3K_4c^4}. \tag{A8}$$

The average occupancy number $\bar{n}$ now includes the contributions from the new states with three and four ions, and can be written as

$$\bar{n}(c) = \sum_{n=0}^{4} np_n = \frac{K_1c + 2K_1K_2c^2 + 3K_1K_2K_3c^3 + 4K_1K_2K_3K_4c^4}{1 + K_1c + K_1K_2c^2 + K_1K_2K_3c^3 + K_1K_2K_3K_4c^4}. \tag{A9}$$

As with $k_{10}$ and $k_{21}$ ($k_{10} < k_{21}$), due to the electrostatic repulsion of the ions inside the filter, we expect $k_{43} > k_{32} > k_{21}$ and $k_{34} < k_{23} < k_{12}$. This gives the inequalities: $K_4 < K_3 < K_2$.

In the range of $c$ where $K_3c \ll 1$, we have $p_4 < p_3 \ll p_2$. Thus, for this range of $c$ we can neglect the values of $p_3$ and $p_4$ in determining the average occupancy number $\bar{n}$. We also find that Eqs. (A4)–(A6) reduce to Eqs. (12)–(14). But, at large enough $c$, the contribution of the $K_1K_2K_3c^3$ and $K_1K_2K_3K_4c^4$ terms in Eqs. (A4)–(A6) may not be negligible, and the quantities $p_3$ (A7) and $p_4$ (A8) will have to be considered in the evaluation of $\bar{n}$, i.e., Eq. (A9) has to be used. However, at these large concentrations, the assumption of constant $u_{eff}$ also may not be valid, so that variation of $u_{eff}$ with $c$ may need to be taken into account. One aspect of this is discussed in Appendix B.

## APPENDIX B: THROUGHPUT CYCLE CONDUCTION MECHANISM

In Appendix A we considered the effects of including the three- and four-ion states of the selectivity filter on the average occupancy $\bar{n}$ of the filter, which were neglected in the derivation of Eq. (17) for the conductance. This equation also assumes that $\bar{n}$ is the only concentration-dependent quantity, i.e., that we can neglect the concentration dependence of the effective electrical mobility $u_{eff}(c)$ in Eq. (16). Here we discuss one of the conditions necessary for the validity of this assumption.

Studying KcsA, Morais-Cabral *et al.* [3] concluded that at external ion concentrations greater than 50 mM the occupancy and ion distribution in the selectivity filter of KcsA do not change much. They found that at these concentrations on average there are two potassium ions in the filter which are evenly distributed over the ion binding sites. They proposed that the increase of the conductance with ion concentration under these conditions occurs due to a throughput cycle (or knock-off) conduction mechanism, whereby the entry of a third ion into the filter is accompanied by a shift of the positions of two filter ions and by an exit of one of them. This mechanism does not require an increase of $\bar{n}$ to increase the conductance and therefore is connected with an increase of the effective electrical mobility $u_{eff}$ with ion concentration.

One can show that the contribution to the channel conductance due to this throughput cycle mechanism ($\Delta g$) can be written as

$$\Delta g(c) = cb_{25}p_{25} + cb_{22}p_{22} = cp_2(b_{25}p_5^{(2)} + b_{22}p_2^{(2)}). \tag{B1}$$

Here $b_{25}$ and $b_{22}$ are proportional to the rate constants for forward and backward equilibrium throughput ion transfers in the doubly occupied filter and are essentially the derivatives of the (nonequilibrium) steady-state rate constants with respect to the applied voltage at a steady-state ion transfer. These rate constants reflect three-ion interactions and are of essentially the same nature as the rate constants for transitions between two- and three-ion states. One of the assumptions for the validity of Eq. (B1) is fast equilibrium transitions between states (25) and (22); this requires both a small energy difference and a small energy barrier between these states. In this case the only rate-limiting step for the throughput cycle mechanism is an entry of the third ion to the filter.

According to Eq. (B1), $\Delta g$ depends linearly on the conditional probabilities $p_5^{(2)}$ and $p_2^{(2)}$ of the states (25) and (22). As was discussed earlier (Sec. II), fast internal transitions lead to the concentration independence of the conditional probabilities. In this case, the concentration dependence of $\Delta g$ is determined solely by the product of $c$ and $p_2(c)$ and thus, in general, is a ratio of a cubic and a quartic polynomial [see Eq. (A6)]. Therefore $\Delta g$ contributes at most over a finite range of $c$. If the equilibrium constants $K_3$ and $K_4$ are small (compared to $K_1$ and $K_2$), such that the probabilities of the three- and four-ion states $p_3(c)$ and $p_4(c)$ are small for not too high $c$, but $c$ is high enough for $p_2$ to be at its (broad) maximum value, $\Delta g$ can exhibit quasilinear behavior with $c$ [3,39]. If, however, for some reason the contributions of $p_{22}$

and $p_{25}$ to $p_2$ are small, the contribution of $\Delta g$ to $g$ will be small too. This probably occurs for low-conductance potassium channels for which the absence of a deep electrostatic well (as occurs in KcsA) and ion-ion repulsion make the state (23) the dominant state for double ion occupancy ($p_2^{(2)}, p_5^{(2)} \ll p_3^{(2)}$). For this state the entry barriers for the third ion are too high, and therefore this state's contribution to the throughput cycle conduction mechanism (B1) can be neglected. This is fortunate for the analysis of Kir2.1 and other low-conductance potassium channels; using Eq. (17) alone involves three parameters, but using Eq. (17) together with Eq. (B1) involves four additional parameters.

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