

Ion conduction in the KcsA potassium channel analyzed with a minimal kinetic model

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We use a model by Nelson to study the current-voltage and conductance-concentration curves of bacterial potassium channel KcsA without assuming rapid ion translocation. Ion association to the channel filter is rate controlling at low concentrations, but dissociation and transport in the filter can limit conduction at high concentration for ions other than K^+ . The absolute values of the effective rate constants are tentative but the relative changes in these constants needed to qualitatively explain the experiments should be of significance.

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Ion channels allow selective transport across cell membranes [1] and KcsA, a bacterial potassium channel of known structure showing high conduction rates and selectivity among monovalent cations, has received much experimental attention recently [2–8]. Theoretical studies using molecular dynamics [9–11] provide atomistic level pictures that can relate structural information to experiments but they involve usually time scales too short to obtain macroscopic currents [10] (except for Brownian dynamics [12]). On the other hand, low-resolution approaches based on continuum [13] and kinetic [14] models introduce severe simplifications concerning structural aspects [14] and channel states and transitions [13], but they provide useful information since electrophysiological experiments are conducted over long times compared to those on an atomic scale [13]. Nelson has recently proposed a kinetic model for ion permeation across open ion channels [15–17]. All microscopic configurations of the channel were represented by only three states that correspond to different ion occupancies of the selectivity filter. When analyzing the experimental data, it was assumed that ion association to and dissociation from the KcsA selectivity filter (and not ion translocation within this filter) could be rate limiting. We use this model to provide explanations for the experimental current-voltage J - V and conductance-concentration g - S curves obtained for a series of monovalent ions by Miller and co-workers [4]. The three-parameter model employed here is distinct from the three-parameter model used by Nelson, although it is a mathematical simplification of the more general four-parameter model discussed in the Appendix [15]. However, we must emphasize the following differences. First, in contrast to Ref. [17] we do not assume rapid ion translocation since this could be rate limiting for ions other than K^+ , as we will show later. Second, for the sake of simplicity, we do not allow the electrical distance δ , an empirical parameter of kinetic models that controls the exponential voltage dependence of the dissociation rate constants, to vary with the ion [17]. Electrical distances give valuable information [1] but they are not free of criticisms [13]. In the case of K^+ , the distances obtained when fitting theory to experiment are reasonable [16,17] but preliminary fittings (see Table 1 in Ref. [17]) gave $\delta=0.3$ for Rb^+ , 0.15

for K^+ , and 0.01 for Tl^+ . These distances differ by a factor of 30 for the narrow, 1.2 nm long selectivity filter [7]. Moreover, the value of δ for Rb^+ is close to that of K^+ and significantly different from that of Tl^+ while the experimental J - V curves are sublinear for K^+ and Tl^+ but superlinear for Rb^+ [4,17]. Electrical distances could not have a simple structural interpretation for the case of concerted multi-ion transport [18]. We have assumed that $\delta=0$ for all ions as a first approximation, and based our analysis on the relative values of the ionic rate constants needed to interpret the experimental data.

Figure 1 shows the conduction steps in the selectivity filter [15]. According to experimental and simulation studies [2,3,6,10], two K^+ ions separated by a single water molecule shift back and forth between the inner (i) and outer (o) configurations. The incorporation of a third ion from the solution causes the exit of a different ion from the opposite side. Simulation studies show that repulsive forces are essential for high conduction rates [11] (they allow the affinity that each ion has for its binding site to be overcome [8]). When the system is in the transition state n , an ion can leave the filter. Ion association to the filter in states o and i is rate controlling at low concentrations but dissociation from the filter in state n and the translocation step between states i and o could limit conduction at high concentrations for ions other than K^+ . Note that introducing $\delta=0$ is equivalent to assuming that neither the association nor the dissociation rate constants are voltage dependent. This assumption could not be valid for quantitative studies [15,17], but it allows us to show the role of the translocation rate constant using a minimum number of model parameters. The states of Fig. 1 incorporate

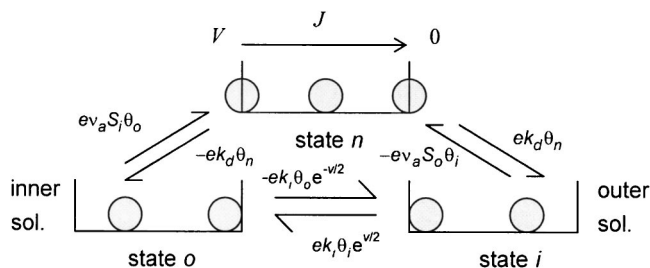


FIG. 1. Schematic view of the conduction steps in the ion channel according to Nelson [15] (we do not assume rapid ion translocation and introduce $\delta=0$).

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the central elements of the channel [3,8], although the vestibule outside the selectivity filter may be included in more refined models [2,5,10,11]. Experiments and simulations show that conformational changes and fluctuations [7,10] can also be important, but the changes in the filter structure at low K^+ concentration are not likely to occur in the high-concentration conduction cycle [7,8]. According to the model of Fig. 1, the ion currents J can be obtained from the system of equations

$$J = e v_a S_i \theta_o - e k_d \theta_n, \quad (1a)$$

$$J = e k_d \theta_n - e v_a S_o \theta_i, \quad (1b)$$

$$J = e k_t e^{v/2} \theta_i - e k_t e^{-v/2} \theta_o. \quad (1c)$$

The probabilities of finding the channel in the states of Fig. 1 are related by $\theta_i + \theta_o + \theta_n = 1$ with $v = eV/kT$ the dimensionless transmembrane voltage, where k is the Boltzmann constant, T is the absolute temperature, and e is the proton charge. Most of the applied voltage falls across the filter [2,18,19], and V is then the potential difference between the filter ends approximately. The ion concentrations are $S_i = S_o = S$ for symmetrical solutions, and v_a , k_d , and k_t are the voltage-independent association, dissociation, and translocation rate constants, respectively. The association rate constant includes both transport (diffusion to the filter) and sorption (dehydration) limitations. The energy landscape and structural periodicity experienced by each particular ion-water queue in the filter [3,7] are lumped into a single translocation rate constant k_t . Note, however, that if the translocation step in Fig. 1 applied only to K^+ but not to other ions (e.g., Rb^+), k_t would not then be the translocation rate constant between configurations i and o , but an effective constant characteristic of ionic transport within the selectivity filter instead (as opposed to the association v_a and dissociation k_d rate constants that characterize interfacial transfer phenomena). The J - V curve that results from Eqs. (1a)–(1c) is

$$J = 2e v_a S \sinh(v/2) \left(\frac{k_d}{v_a S + 2k_d} \right) \left(\frac{k_t}{v_a S + 2k_t \cosh(v/2)} \right). \quad (2a)$$

If translocation is not rate limiting ($k_t \gg v_a S$), Eq. (2a) simplifies to

$$J = e k_d \tanh(v/2) \left(\frac{S}{S + (2k_d/v_a)} \right). \quad (2b)$$

If dissociation is not rate limiting ($k_d \gg v_a S$), Eq. (2a) simplifies to

$$J = e k_t \sinh(v/2) \left(\frac{S}{S + (2k_t/v_a) \cosh(v/2)} \right). \quad (2c)$$

Although Eqs. (2b) and (2c) can be transformed into universal Michaelis-Menten functions of a reduced concentration, this is not the case of the more general Eq. (2a) [15,17]. Therefore, since the Michaelis-Menten kinetics is approximately valid for most channels [1,15,17], the extreme limiting case where $k_t \ll v_a S$ and $k_d \ll v_a S$ simultaneously should

not occur. (In this case the probability of the transient state would be unrealistically high, $\theta_n \approx 1 \gg \theta_i \approx \theta_o \approx 0$, and J would then decrease with S rather than increase.) Equation (2b) could be a reasonable approximation for those ions (e.g., K^+) showing rapid, concerted single-file motion in either direction while Eq. (2c) should be more appropriate for other ions (e.g., Rb^+) where the suboptimal structural periodicity of the water-ion queue makes translocation between states o and i of Fig. 1 more difficult [7]. The more general Eq. (2a) may apply to ions having intermediate properties and to those showing rapid translocation within the filter if the concentration is so high that the usual condition for this case, $k_t \gg v_a S$, is not valid. Note finally that the association step is always rate limiting at low concentrations: both Eq. (2b) and Eq. (2c) predict a linear behavior of J with S for low S . To study the different ion conduction modes present in Eqs. (2a)–(2c), we will assign values to v_a , k_d , and k_t for each ion. Experimental studies [3,4] give K^+ currents in the range 1–40 pA for concentrations in the range 20–800 mM and voltages in the range 20–200 mV. These currents are lower for Rb^+ and Tl^+ , and much lower for Na^+ . When fitting the experimental data [4] to his model, Nelson found $v_a = 6 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ and $k_d = 2 \times 10^8 \text{ s}^{-1}$ for K^+ [17] assuming a voltage dependence to the dissociation rate constants. For the translocation step to be rate limiting, k_d and k_t should take similar values. These values are significant but the rate constants include so many effects (ionic diffusion to the filter, dehydration, translocation between sites, dissociation from the filter, rehydration) lumped into two single numbers that their absolute values are tentative. We will emphasize only the relative changes in the rate constants that are needed to explain qualitatively the J - V curves [4,17] (the same rate constants should be able to describe also the g - S curves [3,4]). Therefore, reference to a particular ion in the calculations is made only to emphasize the qualitative differences between curves. Quantitative studies would need more elaborated models with a higher number of channel states [3] and parameters [17].

Figure 2 shows the J - V curves obtained for K^+ at different concentrations using Eq. (2b) with $v_a = 16 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ and $k_d = 4 \times 10^8 \text{ s}^{-1}$. The dashed curve corresponds to conduction when both ion dissociation and translocation are rate limiting [Eq. (2a) with $k_t = k_d$]. This could be the case of ions undergoing rapid translocation if the concentration is so high that Eq. (2b) is not a good approximation to Eq. (2a), although the ionic fluxes obtained with this equation could not be representative of the channel behavior in the limiting case where $k_t \ll v_a S$ and $k_d \ll v_a S$ simultaneously. The results of Fig. 2 can be compared with the experimental data in Figs. 1 and 2 of Ref. [4]. Although the ability of the filter to show concerted, K^+ single-file motion in either direction may suggest the absence of rectification effects, asymmetric open channel J - V curves could still result from differences between the water-filled central cavity and the channel side bathed by the extracellular solution [2,3]. Rectification effects can be described by introducing asymmetric values to v_a and k_d in Eqs. (1a) and (1b) [17], but these effects could also be due to an asymmetric distribution of the fixed charges in the channel regions adjacent to the selectivity filter ends [6]. This would lead to asymmetric concentrations and pH

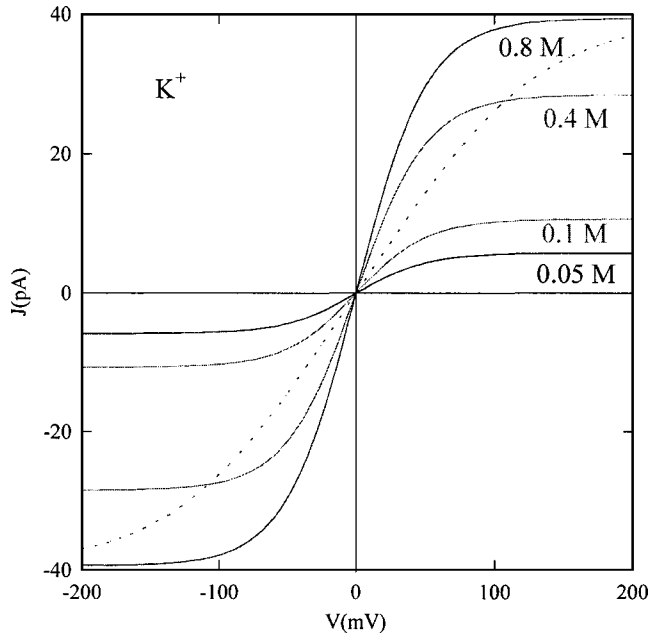


FIG. 2. J - V curves for K^+ symmetrical solutions of different concentration S obtained from Eq. (2b) with $\nu_a = 16 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ and $k_d = 4 \times 10^8 \text{ s}^{-1}$. The dashed curve with $S = 0.8M$ corresponds to conduction when both ion dissociation and translocation are rate limiting [Eq. (2a) with $k_t = k_d$].

values, different from those in the external solutions. It is noteworthy that some channels which have a similar filter to KcsA show different rectification properties [17]. In any case, the relatively weak outward rectification effects observed [4] will not be addressed here using an asymmetric model because they could be influenced by unresolved fluctuations at negative potentials, not being representative of steady-state open channel currents [4,17].

Figure 3 shows theoretical J - V curves for different sets of rate constants intended to be representative of ions K^+ , Tl^+ , Rb^+ , and Na^+ in symmetrical solutions of concentration $S = 0.1M$. The K^+ curve is obtained from Eq. (2b) as in Fig. 2. The Tl^+ curve is also obtained from Eq. (2b), but now with $\nu_a(Tl^+) = \nu_a(K^+)$ and $k_d(Tl^+) = 0.1k_d(K^+)$, since Tl^+ is a tightly binding ion [7] that should not be able to leave the filter as readily as K^+ does. In contrast to the case of Tl^+ where the structural periodicity of the ion-water queue appears to follow closely that of K^+ , the periodicity is suboptimal in the case of Rb^+ [7], although this ion can also enter the filter. Therefore, translocation within the filter can now be rate limiting, and the Rb^+ curve is obtained using Eq. (2c) with $\nu_a(Rb^+) = \nu_a(K^+)$ and $k_t(Rb^+) = 0.005k_d(K^+)$. It should also be noted that Eq. (2c) switches from superlinear to sublinear behavior at very high voltages, in agreement with recent experimental data [5] that show voltage-independent currents suggesting diffusion-limited transport when $V > 300 \text{ mV}$. The J - V curves for Na^+ are also obtained from Eq. (2c) with $\nu_a(Na^+) = 0.01\nu_a(K^+)$ and $k_t(Na^+) = 0.0005k_d(K^+)$ to account for the fact that K^+ transport rates exceed those of Na^+ by several orders of magnitude [4,8]. This gives approximately $J(Na^+) \approx 0.01J(K^+)$ in Fig. 3, which can be decreased further by decreasing $\nu_a(Na^+)$. Na^+ would also show negligible

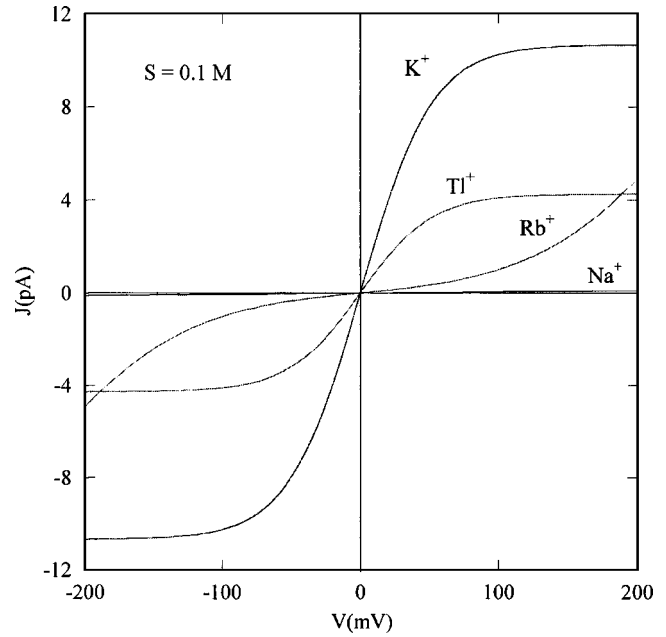


FIG. 3. J - V curves for K^+ , Tl^+ , Rb^+ , and Na^+ in symmetrical solutions of ionic concentration $S = 0.1M$.

transport rates if we used Eq. (2b) instead of Eq. (2c) provided that $k_d \approx k_t$, although it is clear that the two conduction mechanisms are qualitatively different. We have used Eq. (2c) instead of Eq. (2b) here because recent molecular dynamics simulations show that in contrast to K^+ , Na^+ does not exhibit rapid translocation [10]. Figure 3 is in qualitative agreement with the experimental data in Fig. 4 of Ref. [4]: the theoretical results correctly describe the observed superlinear (Rb^+) and sublinear (Tl^+) J - V curves using mecha-

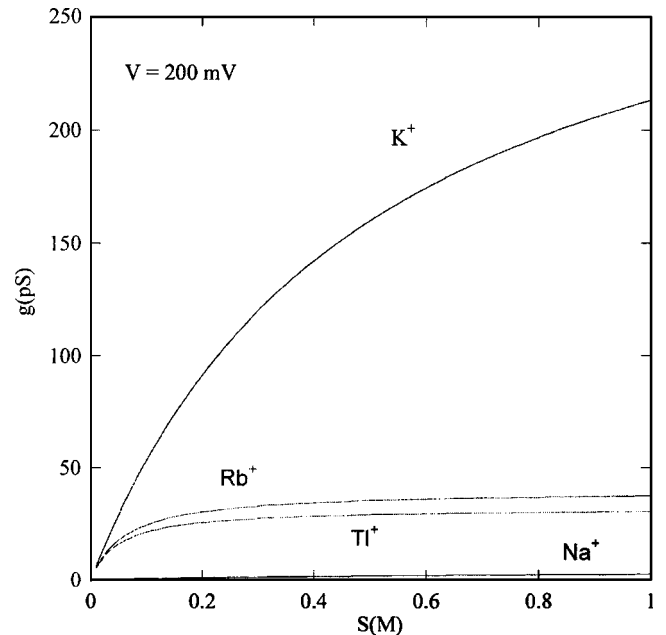


FIG. 4. g - S curves for K^+ , Tl^+ , Rb^+ , and Na^+ in symmetrical solutions of ionic concentration S at transmembrane voltage $V = 200 \text{ mV}$ for the same rate constants of Fig. 3.

nisms for ion conduction mentioned in previous experimental studies [4,3,7,8]. In particular, it is not necessary to assume that the dissociation step is rate limiting to explain the apparently surprising Rb^+ superlinear curve [17] if we consider that it is the translocation step (or the equivalent process characteristic of ionic transport within the selectivity filter for this ion) that limits conduction. Interestingly, a recent experimental study [5] analyzed the effects of Na^+ block on K^+ and Rb^+ currents through single KcsA channels and concluded that the different blocking behavior observed can be correlated with the inhomogeneous energetic landscape of Rb^+ in the selectivity filter. The capability of K^+ to switch from the o and i states in Fig. 1, in contrast to Rb^+ , could explain the marked voltage dependence of Na^+ block in K^+ compared with Rb^+ (see Figs. 3 and 5 of Ref. [5]) although the voltage-dependent kinetics is more complex in this case than that assumed in the present calculations [5]. The dashed curve of Fig. 2 clearly shows that decreasing the value of k_t causes the K^+ current to decrease significantly. At low voltages ($V < 100$ mV in the experiments of Ref. [5]), Na^+ is not able to enter the filter and therefore the J - V curve is similar to that in absence of the blocking ion. However, at higher voltages ($100 < V < 200$ mV) inclusion of Na^+ within the filter disturbs the concerted K^+ translocation between the i and o configurations. This should decrease the effective value of k_t and, according to Eq. (2a), give low values of J . For high voltages ($V > 200$ mV in the experiments of Ref. [5]), Na^+

could be forced through the selectivity filter [5] and the clearance of Na^+ would resume concerted K^+ translocation again with the concomitant increases of k_t and the K^+ current.

Figure 4 shows the g - S curves for ions K^+ , Tl^+ , Rb^+ , and Na^+ in symmetrical solutions of concentration S at a transmembrane voltage $V=200$ mV with the same rate constants as in Fig. 3. There is a rapid increase of conductance at low concentrations that is more evident for K^+ than for the other ions, followed by a tendency to saturation at high concentration. Figure 4 agrees qualitatively with the experimental data in Figs. 3 and 5 of Ref. [4]. Traditional continuum theories based on the Nernst-Planck equation with the Goldman constant field assumption [1] could not deal easily with the single file, concerted multi-ion transport in the filter, and they would naturally lead to curves more linear than those of Figs. 3 and 4, although introduction of additional refinements can significantly improve the theoretical predictions [13]. In the present model, Eq. (2a) gives J - V curves that are intermediate between the sub- and superlinear curves of Eqs. (2b) and (2c) over a range of voltages if we admit that ion conduction can also be limited by transport within the filter (see dashed curve in Fig. 2). In conclusion, although the limited number of microscopic characteristics included in the model precludes its application to a particular set of experimental data, the relative changes needed to explain the qualitative features of the curves for each particular ion are of significance.

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- [1] B. Hille, *Ion Channels of Excitable Membranes*, 3rd ed. (Sinauer Associates, Sunderland, MA, 2001).
- [2] D. A. Doyle, J. H. Morais-Cabral, R. A. Pfuetzner, A. Kuo, J. M. Gulbis, S. L. Cohen, B. T. Chait, and R. Mackinnon, *Science* **280**, 69 (1998).
- [3] J. H. Morais-Cabral, Y. Zhou, and R. Mackinnon, *Nature (London)* **414**, 37 (2001).
- [4] M. LeMasurier, L. Heginbotham, and C. Miller, *J. Gen. Physiol.* **118**, 303 (2001).
- [5] C. M. Nimigean and C. Miller, *J. Gen. Physiol.* **120**, 323 (2002).
- [6] C. M. Nimigean, J. S. Chappie, and C. Miller, *Biochemistry* **42**, 9263 (2003).
- [7] Y. Zhou and R. Mackinnon, *J. Mol. Biol.* **333**, 965 (2003).
- [8] R. Mackinnon, *FEBS Lett.* **555**, 62 (2003).
- [9] J. Aqvist and V. Luzhkov, *Nature (London)* **404**, 881 (2000).
- [10] I. H. Shrivastava, D. P. Tieleman, P. C. Biggin, and M. S. P. Sansom, *Biophys. J.* **83**, 633 (2002).
- [11] S. Bernèche and B. Roux, *Nature (London)* **414**, 73 (2001).
- [12] T. W. Allen, S. Kuyucak, and S. H. Chung, *Biophys. J.* **77**, 2502 (1999).
- [13] W. Nonner, D. P. Chen, and B. Eisenberg, *J. Gen. Physiol.* **113**, 773 (1999).
- [14] C. Miller, *J. Gen. Physiol.* **113**, 783 (1999).
- [15] P. H. Nelson, *J. Chem. Phys.* **117**, 11396 (2002).
- [16] P. H. Nelson, *J. Chem. Phys.* **119**, 6981 (2003).
- [17] P. H. Nelson, *Phys. Rev. E* **68**, 061908 (2003).
- [18] Y. Jiang and R. Mackinnon, *J. Gen. Physiol.* **115**, 269 (2000).
- [19] Y. Jiang, A. Lee, J. Chen, M. Cadene, B. T. Chait, and R. Mackinnon, *Nature (London)* **417**, 523 (2002).