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RESEARCH ARTICLE

Permeation mechanism of a two-state potassium channel

WANG Xiangqun, ZHAO Tongjun (✉), SONG Yang, ZHAN Yong

School of Science, Hebei University of Technology, Tianjin 300130, China

Abstract A two-state hopping model was proposed to study the permeation of ion channel. The Nernst equation in equilibrium and the Michaelis-Menten relation in steady state were derived from the two-state kinetic model. The current-voltage relationship obtained in the symmetrical solutions case was linear when the applied potential was less than 100 mV, which met Ohm's law. The conductance-concentration relationship exhibited the saturation property. Moreover, the characteristic time reaching the steady state of the KcsA channel was also discussed.

Keywords potassium channel, selectivity filter, master equation

1 Introduction

The substances that are very important for vital movement are water miscible in vivo, for example, all kinds of ions and saccharides. These substance need to enter the cell, and water miscible recrement need to expel from the cell. However, the phospholipids bilayer has no permeability for polar molecule or ion. They pass in and out of the cell through the ion channels, which are membrane-spanning proteins. Hydrophilic pores that can selectively mediate ion flux between the outside and inside of the phospholipids bilayer, are essential for the electric activities of the organism, and enable the cell to carry on the necessary transmembrane signal transmission and the physical chemistry regulation between the cells and the external environment under the homeostasis condition (Su, 2000). The most important aspect of the theoretical study on ion channel is to explain the relationship between structure and function. That is, using the structure of the ion channel to illuminate its function characteristic, for example, selectivity, permeability and gating property. While the search for the complete tertiary structure of potassium channels continues, useful insights to the structure of the pore may be obtained from a study on the inverse problem, that is, predicting relevant aspects of the channel structure from its functional property (Chung et al., 2002). There are several theories on ion channel permeation, such as Poisson-Nernst-Planck theory (PNP), Molecular dynamics (MD) model and Brownian dynamics (BD) model (Chung et al., 2002; Chung et al., 2002). Each of them has its merits and shortcomings. The PNP model considers the charge in channel protein, shape of channel, ionic concentration and voltage, but it does not consider the interaction of ion-ion and ion-channel (Graf et al., 2000). MD is a detailed simulation technique based on interaction between atoms. It was used to determine interaction potentials between atoms, but it is not feasible to model the conductance of a channel. Furthermore, MD simulations are too slow at present to determine the channel conductance, which is the most important functional property of an ion channel (An et al., 2005). BD is based on the Langevin equation. It has the ability to calculate the conductance, but it must base on the numerical value of MD simulations.

The three methods consider the course of ionic permeation as a continuous diffusion process. This is a continuous view. From the latest X-ray and molecular dynamics study on KcsA (K^+ conduction and selectivity architecture) channel discovered by MacKinnon's group from the Streptomyces lividans in 2001, there are four discrete potential ion-binding sites in the selectivity filter of KcsA channel. Ion conduction involves transitions between two main states, with two and three K^+ ions occupying the selectivity filter, respectively. Experiment fact suggests there is a series of discrete states in the course of ionic permeation. With the discrete viewpoint on the permeation mechanism, the theoretics is simpler and the image is much clearer.

2 The two-state hopping model and the master equation theory

In 2001, MacKinnon's group presented an improved structure, at a sharp 2 Å resolution, of the Streptomyces lividans

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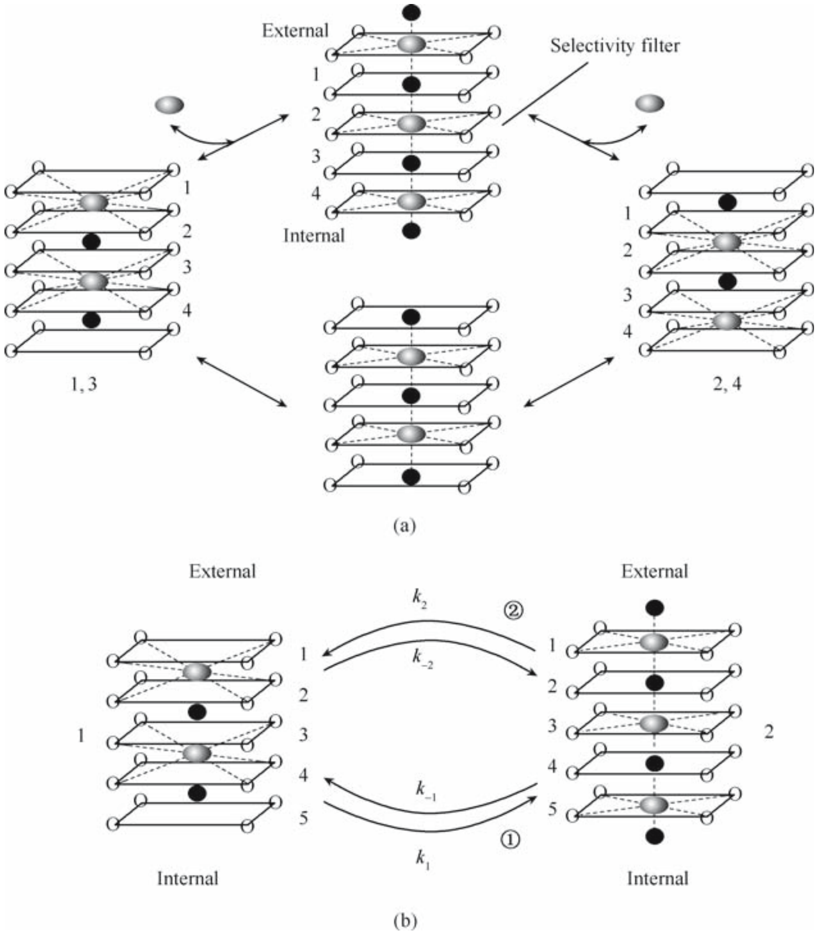
E-mail: zhaotj66@eyou.com

potassium channel with X-ray crystallography (Zhou et al., 2001). MacKinnon and colleagues argue that the four selectivity filter sites are not all occupied simultaneously. Rather, the K$^+$ selectivity filter usually contains two resident K$^+$ ions separated by a water molecule. We defined these sites as positions 1−4, from external side to internal side. The ion pair moves back and forth in a concerted manner between the two configurations. K$^+$–water–K$^+$–water (1,3 configuration) and water–K$^+$–water–K$^+$ (2,4 configuration), and the exchange between the 1,3 and 2,4 configurations occurs either when the ion pair jumps between configurations (concentration-independent path) or when a third ion enters on one side causing an ion to exit from the opposite side (concentration-dependent path) (Fig. 1a) (Morais-Cabral et al., 2001). For K$^+$, the energy difference between the 1,3 and 2,4 configurations is close to zero. The exchange between the two configurations is assumed to be so rapid that we can ignore the time of their translocation. On the basis of the description above, somebody proposed a three- state hopping model. We noticed that there are two states in the selectivity filter: two K$^+$ ion state and three K$^+$ ion state. All the 1,3 and 2,4 configurations contain two K$^+$ ion and don't involve the ion passing in and out of the cell. They have no obvious relation to ion concentration or other outside factors. Considering the two states as one state makes the model simpler. Moreover, we expect to get similar results to former work. On the basis of the description above, we proposed a two-state hopping model (Fig. 1b).

Ion conduction involves transitions of two discrete states in a cycle: state 1 and state 2. The two-ion state is denoted state 1 and the three-ion state is denoted state 2. The probability of finding the selectivity filter in i state is represented by P_i, where $i = 1, 2$. There are stochastic forward and backward transitions between arbitrary adjacent states and the transition rates are described by rate constants $k_{\pm 1}$, $k_{\pm 2}$. There are two paths between the two states: ① and ②. k_1 is dependent on the intracellular concentration $[\mathrm{K}^+]_\mathrm{i}$ and k_{-2} is dependent on the extracellular concentration $[\mathrm{K}^+]_\mathrm{o}$, because transition from state 1 to state 2 needs entering an ion from the intracellular solution, and transition from state 2 to state 1 needs exiting an ion to the extracellular solution. We assume that the total electric potential drop is V from the internal side to the external side of the selectivity filter. According to the Reaction-Rate Theory, all rate constants are voltage-dependent exponentially, namely, become proportional to $e^{\pm\phi}$.



(a)

(b)

Fig. 1 The description of exchange between the 1, 3 and 2, 4 configurations and the schematic view of the two-state hopping model

For convenience, we defined

$$\phi = \frac{\delta N_A q_e z V}{2RT} \tag{1}$$

where, R is the gas constant, T the absolute temperature, N_A Avogadro' number, q_e the elementary charge, z the valence of the permeation ions, δ the equivalent fraction of the total potential drop V.

So, the rate constants read as below

$$\begin{cases} k_1 = v[\mathrm{K}^+]_\mathrm{i}\, e^{\phi} \\ k_{-1} = k_\alpha e^{-\phi} \\ k_2 = k_\alpha e^{\phi} \\ k_{-2} = v[\mathrm{K}^+]_\mathrm{o}\, e^{-\phi} \end{cases} \tag{2}$$

here, the prefactors v, k_α independent on voltage and concentration, are proportional constants to be certain and characteristic for the transitions between the states.

The probability of each state can be described by the following master equations

$$\begin{cases} \dfrac{dP_1}{dt} = -\left(k_1 + k_{-2}\right)P_1 + \left(k_2 + k_{-1}\right)P_2 \\ \dfrac{dP_2}{dt} = \left(k_1 + k_{-2}\right)P_1 - \left(k_2 + k_{-1}\right)P_2 \end{cases} \tag{3}$$

and the normalization is

$$P_1 + P_2 = 1 \tag{4}$$

The net flux J_i across the i state is the difference of two unidirectional fluxes. It can be described as the following

$$\begin{cases} J_1 = k_1 P_1 - k_{-1} P_2 \\ J_2 = k_{-2} P_1 - k_2 P_2 \end{cases} \tag{5}$$

3 Steady-state results

3.1 The Nernst equation at equilibrium-state

At equilibrium, the electrochemical potential across the membrane is zero. So the net flux across each state is zero as well, that is

$$J = J_1 = J_2 = 0 \tag{6}$$

Then

$$\begin{cases} k_1 P_1 - k_{-1} P_2 = 0 \\ k_{-2} P_1 - k_2 P_2 = 0 \end{cases} \tag{7}$$

So

$$\begin{cases} P_1 / P_2 = k_{-1} / k_1 \\ P_2 / P_1 = k_2 / k_{-2} \end{cases} \tag{8}$$

Substituting the constants from equation (2) into the right side of the above equation and multiply together, we can find

$$\begin{cases} P_1 / P_2 = k_\alpha e^{-\phi} / \left(v[\mathrm{K}^+]_\mathrm{i}\, e^{\phi}\right) \\ P_2 / P_1 = k_\alpha e^{\phi} / \left(v[\mathrm{K}^+]_\mathrm{o}\, e^{-\phi}\right) \end{cases} \tag{9}$$

And then, we can find the well-known relationship

$$V = \frac{RT}{2N_A q_e z \delta} \ln \frac{[\mathrm{K}^+]_\mathrm{o}}{[\mathrm{K}^+]_\mathrm{i}} \tag{10}$$

This is the Nernst equation at equilibrium-state (Nelson, 2002).

3.2 Michaelis-Menten kinetics

In steady state, the probability of each state is invariable, thus

$$\frac{dP_i}{dt} = 0 \ (i = 1, 2) \tag{11}$$

The master equation (3) becomes

$$\left(k_1 + k_{-2}\right)P_1 = \left(k_2 + k_{-1}\right)P_2 \tag{12}$$

From the definition of the net flux equation (5), the above equation (12) indicates that all the net fluxes of the two states are equal

$$J = J_1 = J_2 \tag{13}$$

From equation (12), the probabilities P_1, P_2 can be solved analytically and all the two probabilities are functions of the rate constants. Substitute the results of the two probabilities P_1, P_2 into equation (5), and the net flux becomes

$$J = \frac{k_1 k_2 - k_{-1} k_{-2}}{k_1 + k_2 + k_{-1} + k_{-2}} \tag{14}$$

where all the rate constants are dependent on voltage. In addition, the two rate constants k_1 and k_2 are relevant to intracellular concentration $[\mathrm{K}^+]_\mathrm{i}$ and extracellular concentration $[\mathrm{K}^+]_\mathrm{o}$ respectively. When ions are present only on the outside, the net flux simplifies into

$$J = \frac{-k_{-1}}{\dfrac{\left(k_2 + k_{-1}\right)e^{\phi} / v}{[\mathrm{K}^+]_\mathrm{o}} + 1} \tag{15}$$

This is identical to the saturating function

$$J(V) = \frac{J_{\max}(V)}{1 + K_s(V) / [\mathrm{K}^+]_\mathrm{o}} \tag{16}$$

here the saturation net flux $J_{\max}$ and the Michaelis-Menten coefficient K_s are given by

$$\begin{cases} J_{\max}(V) = -k_{-1} \\ K_s(V) = (k_2 + k_{-1})e^{\phi}/v \end{cases} \tag{17}$$

Since, the rate constants k_2, k_{-1} are only functions of voltage V, so do $J_{\max}$ and K_s. Because the current across the channel can be given by

$$I = zq_e J \tag{18}$$

From equations (16) and (18), we can find

$$I(V) = \frac{I_{\max}(V)}{1 + K_s(V)/[\mathrm{K}^+]_o} \tag{19}$$

This is the Michaelis-Menten kinetics relationship of ion channel (Nelson, 2002).Where

$$I_{\max} = q_e J_{\max} \tag{20}$$

At any fixed voltage, the ion current varies with concentration according to the Michaelis-Menten equation (18) which was observed experimentally. From this equation, we can see that the relation between current and ion concentration is linear when $[\mathrm{K}^+]_o << \mathrm{K}_s$, however, the current approaches the saturation current I_{max} when $[\mathrm{K}^+]_o >> \mathrm{K}_s$.

3.3 Conductance-voltage relationship

The K^+ conductance in KcsA channel in the two-state hopping model are calculated when the channel is exposed to symmetric solutions $[\mathrm{K}^+]_o = [\mathrm{K}^+]_i = [\mathrm{K}^+]$ and the absolute temperature we used is 298.15 K. The concentrations of the K^+ in the calculation are 250, 500, 750 mmol, 1 and 1.5 mol respectively and the applied voltage ranging from 100 to 500 mV. However, we still need the 3 parameters δ, v and k_α We use the 3 parameters as

$$\begin{cases} \delta = 0.16 \\ v = 5.9\times10^8 \\ k_\alpha = 3.6\times10^8 \end{cases} \tag{21}$$

The first two parameters provided by (Nelson, 2002).

The conductance calculated under different conditions is shown in Fig. 2. Looking at the figure, we can see that all curves are linear when the applied voltage is less than 100 mV. This suggests the conductance-voltage relationship comply with Ohm's law. The conductance-voltage relationship deviates slightly from Ohm's law at higher applied potentials. Therefore, it is feasible for analyzing the conductance- voltage relationship by our two-state hopping model and master equation theory.

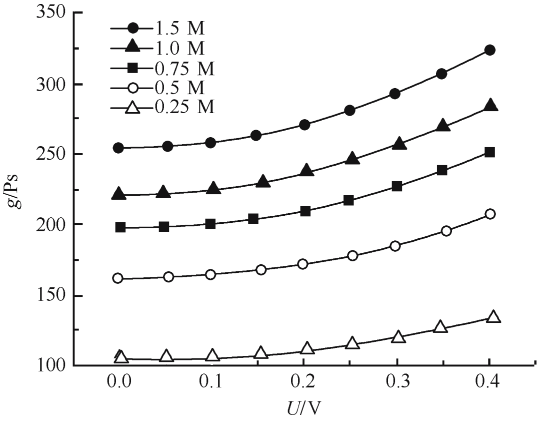


Fig. 2 The g-U relationship for KcsA channel by two-state hopping model in different concentration

3.4 Current-concentration relationship

Experimentally, the current across the potassium channel first increases with an increasing ionic concentration and then saturates.

We study the current properties of potassium ions by performing two-state hopping model under various applied voltages 10, 50, 100, 150 and 200 mV. The current- concentration curves for the channels with the concentration ranges from 0 mol to 3 mol (Fig. 3). From the figure, we can see that the current increased linearly with an increasing ionic concentration at first and then began to saturate. The current-concentration relationship complies with the Michaelis-Menten kinetics equation. Experimentally the range of K_s is 40–300 mmol (Chung et al., 2002) and our theoretical value of K_s is 250–300 mmol, which suggests the validity of our two-state hopping model in this channel.

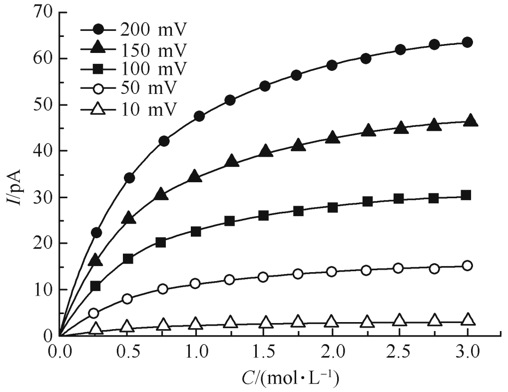


Fig. 3 The current-concentration relationship for KcsA channel by two-state hopping model in different applied voltage

4 The transient characteristic of ion channel

In the following, the transient characteristic for the KcsA channel is discussed. The ion channel will experience a transient course and the probability P_i $(i = 1, 2)$ of each state will change with time t, before it reaches the steady state. After a short time transient, the channel will reach the steady state, which is discussed above.

The time-dependent probability P_i ($i = 1, 2$) of each state can be described as master equation (3) and the normalization (4). We can deduce that each probability meets the following equation:

$$\frac{dP_1}{dt} = -\left(k_1 + k_{-2} + k_{-1} + k_{-2}\right)P_1 + \left(k_2 + k_{-1}\right) \tag{22}$$

For simplicity, we defined

$$A = k_1 + k_2 + k_{-1} + k_{-2}, \quad B = k_2 + k_{-1} \tag{23}$$

Thus, by solving equation (22) we can deduce the probability of each state and the characteristic time τ for the channel to reach the steady state.

$$P_1 = Me^{-t/\tau} + \frac{B}{A} \tag{24}$$

$$\tau = \frac{1}{k_1 + k_2 + k_{-1} + k_{-2}} \tag{25}$$

where M is a constant determined by initial condition.

Because rate constants are relevant to voltage, concentration and temperature, and because characteristic time has a relationship with rate constants, characteristic time is relevant to voltage, concentration and temperature. In the following, we will discuss the relationships between characteristic time and voltage, concentration and temperature.

4.1 Characteristic time-voltage relationship

In the following, we will discuss the relationships between characteristic time and voltage in the stated temperature. The applied voltage ranging from 0 to 400 mV and other conditions keep stated. The curves are shown in Fig. 4. From the curves, we can see that the characteristic time decreases with the increase of both the applied voltage and the ionic concentration. Under different given voltage and concentration, the time constant is less than the ion transferring time 10 ns (Xu et al., 2004). This indicates that the transient characteristic of the ion channel is extremely short.

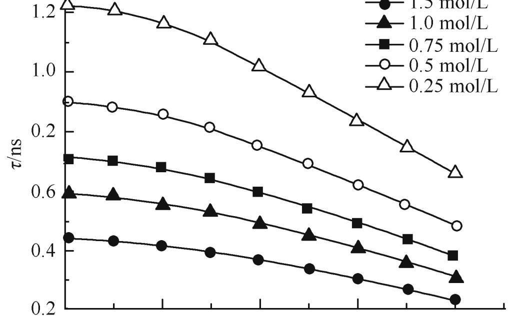


Fig. 4 The τ-U relationship for KcsA channel by two-state hopping model in different concentration

4.2 Characteristic time-concentration relationship

We study the characteristic time-concentration relationships under various applied voltages 10, 50, 100, 150 and 200. The characteristic time-concentration curves for the channels with the concentration ranging from 0 mol to 2 mol are shown in Fig. 5. From the figure, we can see that the characteristic time rapidly decreases with the increase of the ionic concentration, when the applied voltages are kept constant. The characteristic time rapidly decreases with the increase of the applied voltage also, when the ionic concentration is kept constant.

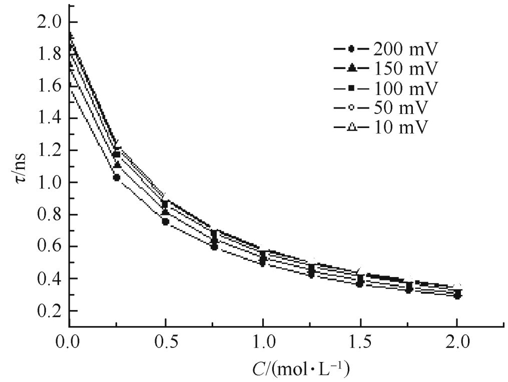


Fig. 5 The τ-C relationship for KcsA channel by two-state hopping model in different applied voltage

4.3 Characteristic time-temperature relationship

Figure 6 is the characteristic time-temperature relationship under stated ionic concentration (0.25 mol) and various applied voltages. The voltages are 10, 50, 100, 150 and 200 mV and temperature ranging from 298.15 to 378.15 K. From these curves, we can see that temperature has an obvious effect when the voltage is high, and there is almost no effect when the voltage is low.

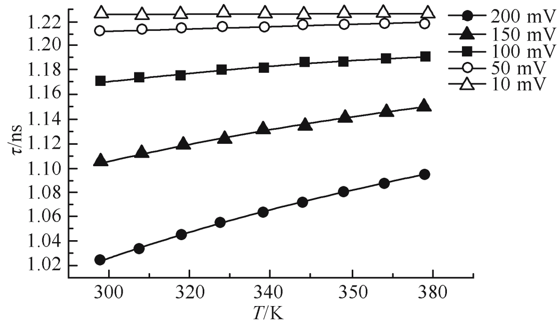


Fig. 6 When concentration is 0.25 mol / L, the τ-T relationship for KcsA channel by two-state hopping model in different applied voltage

5 Conclusions

In this paper we start from the three-dimensional structure of KcsA potassium channel, construct a two-state hopping model, then take two-ion state and three-ion state as two states of our model to study the permeation of KcsA channel. We have deduced some well-known properties of the KcsA potassium channel such as the Nernst equation in equilibrium state and the Michaelis-Menten kinetic relationship in steady state. Further, we display the *g-V* curves and *I-C* curves under different conditions. From the conductance-voltage relationships, we can see that all curves are linear when the applied voltage is less than 100 mV. When the voltage increases, the conductance-voltage relationships deviate from Ohm's law. From the current- concentration relationship curves, we can see that the current increases linearly with an increasing ionic concentration at first and then begins to saturate. The current-concentration relationship complies with the Michaelis-Menten kinetics equation. We can see the availability of our model. In addition, we discussed the transient time to reach the steady state and the relationships between characteristic time and voltage, and concentration and temperature. From these curves, we can see that temperature has obvious effect when the voltage is high, and there is almost no effect when the voltage is low. We studied the permeation mechanism of KcsA potassium channel by using the two-state hopping model and master equation theory. The model is simple and reconcilable parameter is little. However, we still gained some results consistent with experimental results, though our model still needs improvement to inosculate the experimental results.

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