

УДК 577.322

Yesylevsky S. O., Kharkyanen V. N.

GENERAL THEORY OF THE ION CHANNEL WITH MULTIPLE OCCUPANCY

Department of Biophysics, Institute of Physics, Natl. Acad. of Sci. of Ukraine. Prospect Nauky 46, Kyiv-03039, Ukraine, E-mail vakhar@mail.kar.net.

We present a general theoretical approach, which explains the conductance in the ion channel with multiple occupancy. The model is based on the design of KcsA K⁺ channel, but not limited to it. We show that the motion of the ions in the selective filter is concerted and can be reduced to the motion of a single quasi-particle called quasi-ion. The concept of quasi-ions provides an elegant explanation of barrier-less "knock-on" conduction in the selectivity filter and allows us to avoid explicit description of the motion of individual ions in the multi-ion channel. The quasi-ions perform actual charge transfer in the channel.

Key words: Hierarchy of motions, quasi-particles, potassium channel, KcsA channel, selectivity filter.

Introduction

High-resolution x-ray structures of bacterial KcsA K⁺ channel, first published in 1998 [5], brought a new insight into the ion channels science. It was established that the most important feature in KcsA structure is a narrow (~3Å) selective filter. It is responsible for selectivity and conduction [1-4] and possibly for the fast gating of the channels [9-11] and their C-type inactivation [13-14, 16-18]. Up to 3 K⁺ ions separated by the water molecules can be accommodated in the filter [1-4, 15]. Recent molecular dynamics (MD) studies revealed barrierless "knock-on" conduction mechanism in the filter [2]. An exceptional role of ion-ion forces in the energy balance of different ion configurations in the conduction pathway was established [1-4]. Although the location of the fast gate in the selectivity filter region may be considered established, the molecular mechanisms responsible for gating are still not well characterized. It is well known that the ions in the selectivity filter interact with the pore walls strongly [1-6]. Ions create very strong local electrostatic field, which can substantially alter the conformation and polarization state of the filter walls. Such large-scale changes are likely to be much slower than the time of single ion passing and thus will "feel" the mean occupancy of the filter. In our previous works [21]

we show that such systems with strong ion-conformational interaction (ICI) may exhibit complex self-organized behavior. Particularly two steady states, which correspond to the closed and open states of the channel, may appear [21]. This concept is very attractive because it explains the appearance of discrete conductance states from the very basic principles. It is not strongly "bound" to particular molecular structure and is applicable to the broad range of systems [20, 21]. The concept was successfully applied to bacterial reaction centers [21] but its application to the ion channels was long hampered despite the number of experimental proofs [11]. The reason was that the ion channels were recognized to be multi-ion pores, while explicit accounting for multiple ions in ICI leads to intolerable mathematical complications. In the present study we propose a general theory of the multi-ion channels, which allow us to model the behavior of several strongly interacting ions in the wide energy well. It allows to reduce the motion of multiple ions to the motion of a single quasi-particle. This greatly simplifies the problem and allows us to use the ICI concept.

In the present work we formulate our approach in terms of general single-ion energy profiles and ion-ion interaction forces. We show that in certain cases it is possible to consider the fast fluctuations of the ion-ion distances to be adiabatically decoupled.

led from the slow motion of the ions' center of masses. The latter can be considered as a quasi-particle called a quasi-ion. Subsequent development includes modeling of the ion exchange with solutions and description of the conduction in terms of quasi-ions.

Conduction in the ion channel with multiple occupancy

The character of the ions motion in the filter

Although selective filters of the K^+ channels are long recognized to be multi-ion pores, existing theoretical models of conduction seem to underestimate the importance of the collective motions of the ions in the filter. Conventional models of multiply occupied channels treat them as a system of binding sites, which may contain only one ion each. Ion-ion interactions are usually treated implicitly as restrictions that allow one ion to reside in each binding site [21###]. These models are unlikely able to describe the energy profile in the filter adequately. It is believed that the selectivity filter is a rather extended and deep energy well that contains several strongly interacting ions [1-4]. It is necessary to note, that the energy profile in the selectivity filter may contain local minima, which are often considered to be preferable ions locations. However this minima are small in comparison with the full depth of the potential well in the filter [4] and overall dynamics of the ions can not be described as single-ion "jumps". Modern MD and BD simulations allow studying the motion of individual ions in the channel pore explicitly. However, they operate on the time scales much shorter than characteristic time of the ion translocation and thus are "excessively detailed" for the majority of applications. In addition, recent MD studies [2] suggest that permeation through the channel is an essentially collective effect and requires relatively slow concerted motion of all ions in the filter. However, there are no theoretical explanations of this interesting behavior. Thus, a simple theoretical approach, which correctly describes the permeation through the channel but eliminates unnecessary small-scale details, seems to be very attractive.

Energy profiles and motion equations

Let us consider a generalized model of the channel pore that may accommodate several ions at once. The pore is assumed to be axially symmetric

and the ions are considered confined at the pore axis. Thus, the ions are assumed to move in one dimension in a single-file manner.

Single ion energy profile $U(x)$ gives the energy of the solitary ion at the point x on the channel axis. $U(x)$ includes the interaction of the ion with the pore walls and the contribution of the external membrane potential. Relationship between the model pore and the structure of the real channel is explained in Figure.

The energy of the ion-ion interaction is $V(r)$, where r is the distance between the ions. It is assumed that the potential $V(r)$ accounts for the fast components of polarization in the channel protein and water molecules situated between the ions but does not depend on the exact positions of the ions in the pore.

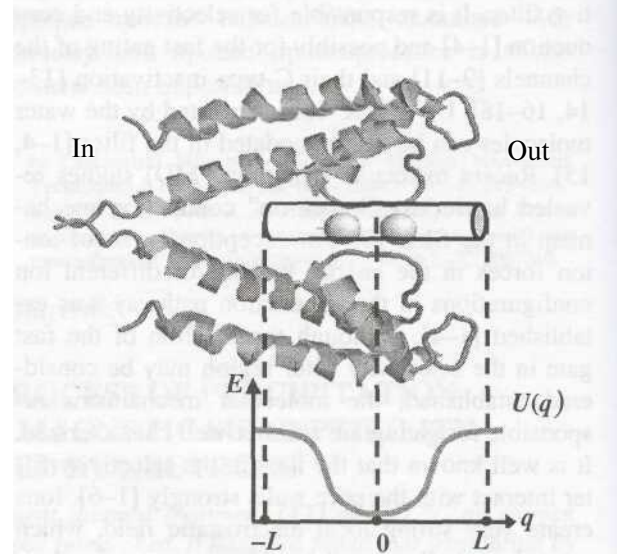
The motion of the ions in the pore is assumed to be overdamped and may be described by the Langevin equations:

$$\dot{x}_i = -\frac{1}{m\gamma} \frac{\partial E(x_i)}{\partial x_i} + F_R(t), \quad (1)$$

where m is the mass of the ion, γ is the friction coefficient, $E(XI)$ is the energy of the i -th ion, F_R is the random force, which correspond to the thermal fluctuations. The friction coefficient can be calculated from the ion's diffusion coefficient D as $\gamma = k_B T / mD$.

We model the random force as a white noise $\xi(t)$

$$\begin{aligned} \langle \xi(t) \rangle &= 0, \\ \langle \xi(t) \cdot \xi(s) \rangle &= \delta(t - s). \end{aligned} \quad (2)$$



Relationship between the real structure of KcsA potassium channel and the model pore. KcsA channel is shown with two K^+ ions in the selectivity filter. Only two of four subunits are shown for clarity. The cartoon of the model pore is superimposed on the selectivity filter. Dimensions of the model pore and the example of the single ion energy profile are shown

If the channel contains N ions, then the energy of the z -th ion is

$$E(x_i) = U(x_i) + \sum_{j=1, j \neq i}^N V(x_j - x_i) \quad (3)$$

and equation (1) may be rewritten as

$$\dot{x}_i = -\frac{D}{k_B T} \left(U'(x_i) - \sum_{j=1, j \neq i}^N V'(x_j - x_i) \right) + \sqrt{2D} \xi_i(t), \quad (4)$$

where $U'(x) = \frac{\partial U(x)}{\partial x}$, $V'(r) = \frac{\partial V(r)}{\partial r}$ are the forces acting on the ion from the pore wall and the other ions, respectively.

It is convenient to introduce new variables

$$\begin{aligned} q &= \frac{\sum_{i=1}^N x_i}{N}; \\ p_i &= x_{i+1} - x_i, \quad i = 1, 2, \dots, N-1 \end{aligned} \quad (5)$$

instead of the ions' coordinates x_i , q is the position of the center of masses of all ions in the filter, p_i are the distances between adjacent ions (the ions are sequentially numbered from left to right), N is the number of ions in the channel.

The positions of individual ions are expressed in the terms of new coordinates as

$$x_i = \frac{1}{N} \left(qN + \sum_{j=1}^{i-1} j p_j - \sum_{j=1}^{N-i} j p_{N-j} \right). \quad (6)$$

The distance between two arbitrarily chosen ions i and j ($j > i$) is

$$r_{ij} = x_i - x_j = \sum_{k=i}^{j-1} p_k. \quad (7)$$

Adding and subtracting equations (4) one can obtain the equations for q and p . Using obvious relation $V'(x_i - x_j) = -V'(x_j - x_i)$ one can transform the system of equations (4) to

$$\begin{cases} \dot{q} = -\frac{D}{Nk_B T} \sum_{i=1}^N U'(x_i(q, \vec{p})) + \sqrt{\frac{2D}{N}} \xi(t), \\ \dot{p}_i = -\frac{D}{k_B T} [U'(x_{i+1}(q, \vec{p})) - U'(x_i(q, \vec{p})) + \\ + \sum_{j=1}^N V'(x_j(q, \vec{p}) - x_{i+1}(q, \vec{p})) - \\ - \sum_{j=1}^N V'(x_j(q, \vec{p}) - x_i(q, \vec{p}))] + \sqrt{4D} \xi_i(t), \end{cases} \quad (8)$$

where $p = \{p_1, p_2, \dots, p_{N-1}\}$. There are no terms, which describe the ion-ion interaction in the first equation of (8). This important feature is used below to determine fast and slow motions in the system.

Characteristic times

The motion of the ions in the channel can be characterized by three time constants: τ_q , τ_p and τ_{ex} , τ_q is the relaxation time of q variable. τ_p is the mean relaxation time of all p 's. Finally, τ_{ex} is the mean time between the events of ion exchange between the channel interior and external solutions.

The values of the time constants can be evaluated by considering returning forces F_q and F_p , which drive the system back to the steady state after small perturbation and act on q and p variables, respectively.

The following limiting cases may be considered:

Weak interaction: $F_q \gg F_p$, $\tau_q \ll \tau_p$.

Moderate interaction: $F_q \sim F_p$, $\tau_q \sim \tau_p$.

Strong interaction: $F_q \ll F_p$, $\tau_q \gg \tau_p$.

The weak interaction case is probably not applicable for real channels, however it may be important in the other systems. The moderate interaction case is the most complicated one, because the system of equations (8) or its equivalent (4) should be solved explicitly, which means in fact the Brownian Dynamics simulation. The strong interaction case, however, allows adiabatic decoupling of the fast motions, which are vibrations of the ion-ion distances. This greatly simplifies the problem. Subsequent development is done in the assumption of strong interaction. It will be shown below that the strong interaction is observed in the model of Kcsa selectivity filter.

Quasi-ions

Equations (8) for q may be written as

$$\dot{q} = -\frac{D}{Nk_B T} F(q, \vec{p}) + \sqrt{\frac{2D}{N}} \xi(t), \quad (9)$$

where

$$F(q, \vec{p}) = \sum_{i=1}^N U'(x_i(q, \vec{p})).$$

As it is stated above, in the strong interaction case the relaxation of ion-ion distances is much faster than that of the ions' center of masses. The characteristic time needed for the ion-ion distances to reach the equilibrium distribution is i_p . It is much shorter than the time scale of q relaxation, which is the time scale we are interested in. Thus, it is possible to assume that the distribution of the ion-ion distances is close to the equilibrium distribution $f_p(q, p)$ for each given q :

$$f_0(q, \vec{p}) = \frac{\exp\left(-\sum_{i=1}^N E(x_i(q, \vec{p}))/k_B T\right)}{\int \exp\left(-\sum_{i=1}^N E(x_i(q, \vec{p}))/k_B T\right) d\vec{p}} \quad (10)$$

(see [21]).

On the time scale of q relaxation, there is an "averaged" effective force acting on q , which is equal to

$$\begin{aligned} F_{eff}(q) &= \int F(q, \vec{p}) f_0(q, \vec{p}) d\vec{p} = \\ &= \int \sum_{i=1}^N U'(x_i(q, \vec{p})) f_0(q, \vec{p}) d\vec{p}. \end{aligned} \quad (H)$$

If the introduced adiabatic approximation is valid, then equation (9) can be reduced to

$$\begin{aligned} \dot{q} &= -\frac{D}{Nk_B T} \int \sum_{i=1}^N U'(x_i(q, \vec{p})) f_0(q, \vec{p}) d\vec{p} + \\ &+ \sqrt{\frac{2D}{N}} \xi(t). \end{aligned} \quad (12)$$

See Appendix C for derivation details. Introducing the effective potential for q

$$\frac{\partial U_{eff}(q)}{\partial q} = \int \sum_{i=1}^N U'(x_i(q, \vec{p})) f_0(q, \vec{p}) d\vec{p}, \quad (13)$$

one can obtain final equation for q

$$\dot{q} = -\frac{\tilde{D}_N}{k_B T} \frac{\partial U_{eff}(q)}{\partial q} + \sqrt{2\tilde{D}_N} \xi(t), \quad (14)$$

where $\tilde{D}_N = D/N$. This is essentially the Langevin equation for a particle with a mass of N ion masses that performs stochastic motion in the effective potential U_{eff}

Thus, the motion of the multiple ions in the pore can be reduced to the motion of their center of masses in the case of strong ion-ion interactions. The whole set of ions in the pore can be considered as a quasi-particle, referred to as *quasi-ion* that has the mass of N ion masses and moves in the effective potentials according to the Langevin equation (14).

Modeling ion exchange events

The concept of the quasi-ions is formulated for the channel that permanently contains N ions. However, in real channels the occupancy state is changed abruptly during the events, when the ions are exchanged with the external solutions. Exchange events cannot be directly described in terms

of the quasi-ions because only individual ions can leave or enter the channel. However, it is possible to estimate the probability of the ion exchange events for a given position of the quasi-ion.

Let us assume that the channel contains N ions and the corresponding quasi-ion is located at the point q . The edges of the channel pore are at the points $\pm L$. Positions of the individual ions fluctuate around their local equilibrium values according to the distribution $f_0(q, \vec{p})$ given by (10). The ion can leave the channel only if it comes sufficiently close to the edge of the channel. Let us define these "exit regions" as the narrow "slices" of width Δx at the edges of the channel. The probabilities of finding the ions in the left and right exit regions are

$$\Theta_L^{(N)}(q) = \int_{-L < x_1(q, \vec{p}) < -L + \Delta x} f_0(q, \vec{p}) d\vec{p}, \quad (15)$$

$$\Theta_R^{(N)}(q) = \int_{L - \Delta x < x_N(q, \vec{p}) < L} f_0(q, \vec{p}) d\vec{p}. \quad (16)$$

If the ion appears in the exit region it escapes with the fixed probability k , which is the rate constant for crossing the channel boundary.

The probabilities of entering the channel from the left and right solutions are

$$P_L = kC_L, \quad P_R = kC_R \quad (17)$$

respectively, where C_R and C_L are the effective concentration in the left and right solutions.

At the moment of ion exchange the "dimensionality" of the quasi-ion abruptly changes and it "jumps" to the location of the center of masses of the remaining ions (actually the "new" quasi-ion is not formed instantaneously, but after very short equilibration period comparable to i_p). The new position of the quasi-ion is strictly determined by its position before the exchange event. Let us assume that the channel contained N ions at the instant of exchange event and the ion is crossing the left channel boundary. The first ion is, therefore, in the left exit region and the quasi ion is located at the point

$$q_N = \frac{-L + \sum_{i=2}^N x_i}{N}, \quad (18)$$

where subscript N indicates the current channel occupancy. If the first ion escapes, the remaining $N - 1$ ions form a quasi-ion at the point

$$q_{N-1} = \frac{\sum_{j=1}^{N-1} x_j}{N-1}, \quad (19)$$

where the ions are renumbered in such a way that $j = i-l$. Comparing (18) and (19) we have

$$q_N N + L = q_{N-1} (N-1). \quad (20)$$

If the ion is crossing the right boundary, then

$$q_N N - L = q_{N-1} (N-1). \quad (21)$$

The same relations (20) and (21) describe the entering process when the occupancy state changes from $N-l$ to N .

Current through the channel and the mean occupancy

In order to model the conduction through the channel one should combine the motion of the quasi-ions between the exchange events with the "switches" of the channel occupancy at these moments. Each of the possible occupancy states of the channel is described by the Focker-Planck equation, which corresponds to the Langevin equation (14)

$$\frac{\partial P_N(q,t)}{\partial t} = \frac{D}{N} \frac{\partial}{\partial q} \left(\frac{1}{k_B T} \frac{\partial U_{eff}(q)}{\partial q} P_N(q,t) + \frac{\partial P_N(q,t)}{\partial q} \right),$$

where P_N is the distribution function of the quasi-ion in the occupancy state N .

This equation should be appended by the terms, which describe ion-exchange events according to the scheme $N-l \leftrightarrow N \leftrightarrow N+1$. Using (15), (16), (17), (20) and (21) we derive a quite complicated equation for the distribution function of the quasi-ion, which accounts for the ion-exchange events and is valid for any concentrations of the ions in solutions and arbitrary membrane potentials.

$$\begin{aligned} \frac{\partial P_N(q)}{\partial t} = & \frac{D}{N} \frac{\partial}{\partial q} \left(\frac{1}{k_B T} \frac{\partial U_{eff}(q)}{\partial q} P_N(q) + \frac{\partial P_N(q)}{\partial q} \right) + \\ & + k C_L P_{N-1} \left(\frac{qN+L}{N-1} \right) + k C_R P_{N+1} \left(\frac{qN-L}{N-1} \right) + \\ & + k \Theta_L^{(N+1)} \left(\frac{q(N+1)+L}{N} \right) P_{N+1} \left(\frac{q(N+1)+L}{N} \right) + \\ & + k \Theta_R^{(N+1)} \left(\frac{q(N+1)-L}{N} \right) P_{N+1} \left(\frac{q(N+1)-L}{N} \right) - \\ & - k (C_L + C_R) P_N(q) - k (\Theta_L^{(N)}(q) + \Theta_R^{(N)}(q)) P_N(q) \end{aligned} \quad (22)$$

(see [21] and the references herein for detailed proof). In this equation the first term describes the motion of the quasi-ion of dimensionality N be-

tween the exchange events, terms 2 and 3 correspond to the transition $N-1 \rightarrow N$, terms 3 and 4 to the transition $N+1 \rightarrow N$, terms 5 and 6 to the transitions $N \rightarrow N+1$ and $N \rightarrow N-1$ respectively.

For practical calculations the number of allowed occupancy states can be restricted by the maximally possible occupancy N_{max} , because the states with the high occupancies are very improbable due to the "cluttering" of the ions and, therefore, very high energies of the corresponding states.

The current through the channel in the steady state can be calculated as the integral flux through one of channel edges (say, left).

$$\begin{aligned} I = I_0 \sum_{N=1}^{N_{max}} \int_{-L}^L & \left[k \Theta_L^{(N)}(q) P_N(q) - C_L P_N(q) + \right. \\ & + k \Theta_L^{(N+1)} \left(\frac{q(N+1)+L}{N} \right) P_N(q) - \\ & \left. - k C_L P_N \left(\frac{qN+L}{N-1} \right) \right], \end{aligned} \quad (23)$$

where I_0 is an empirical constant derived from the comparison with experimental or BD data. Two other empirical parameters Ax and k are determined from the comparison of theoretical and BD distributions of the quasi-ions as it described below.

Another important property of the channel is its mean occupancy. It can be calculated as the weighted sum over the occupancy states

$$N_{mean} = \sum_{N=1}^{N_{max}} N \int_{-L}^L P_N(q) dq. \quad (24)$$

Conclusions

We have developed a general theoretical approach, which provides a description of ions' translocation in the channels with multiple occupancy. It is shown that there is a hierarchy of motions in the selective filter of the channel: the fluctuations of the ion-ion distances are much faster than the motion of the ions' center of masses. The latter may be considered as a quasi-particle, called quasi-ion. The quasi-ion moves in the effective potential, which is formed by the pore wall and the fast fluctuations of the ion-ion distances. Our approach allows to reduce the motion of multiple interacting ions to the translocation of single quasi-ion. This simplifies the description of the ion conductance greatly and opens the perspectives for further theoretical development.

1. Zhou Y., Moralis-Cabral J. H., Kaufman A., MacKinnon R. II Nature. - 2001. - 414. - P. 43-48.
2. Berneche S., Roux B. II Ibid. - P. 73-77.
3. Moralis-Cabral J. H., Thou. K., MacKinnon R. II Ibid. - P. 37-42.

- Mashl R. J., Tang Y., Schnitzer J., Jakobsson E. II Biophys. J. - 2001. - 81. - P. 2473-2483.
- Doyle D. A., Cabral J. I. M., Pfuzner R. A. et al. // Science. - 280. - P. 69-77.

6. Chang S., Allen T. W., Hoyles M., Kuyucak S. *II Biophys. J.*- 1999.-77,-P. 2517-2533.
7. Carpenter C. E., Shrivastana I. H., Ranatunga K. M. at al. // *Ibid.*- 78.- P. 2929-2942.
8. Berneche S., Roux B. *II Ibid.*- P. 2900-2917.
9. Karachi Y., Jan L. Y., Lazdunski M. Potassium Ion Channels. Molecular structure, function and diseases. Academic press.- 1999.
10. Proks P., Capener C. E., Jones P., Ashcroft F. M. *II J. Gen. Physiol.*-2001.- 118.-P. 341-353.
11. Choe H., Sackin H., Palmer L. G. *II Ibid.*- 1998.- 112.- p. 433-446.
12. Choe H., Sackin H., Palmer L. G. *II Ibid.*- 2000.- 115.- P. 391-404.
13. Kiss L., LoTurco J., Korn S. J. *II Biophys. J.*- 1999.- 76.- P. 253-263.
14. Demo S. D., Yellen G.- 1991.- 7.- P. 743-753.
15. Lipkind G. M., Hanck D. A., Fozzard H. A. *Proc. Natl. Acad. Sci.*-1995,-92,-P. 9215-9219.
16. Antz C., Geyer M. *II Nature.*- 1997.- 385,- P. 272-274.
17. Lopez-Barneo J., Hoshi T., Heinemann S. H., Aldrich R. W. *II Receptors and Channels.*- 1993- 1.-P. 66-71.
18. Liu Y., Junnan M. E., Yellen G. *II Neuron.*- 1996.- 16.- P. 859-867.
19. Melishchuk A., Loboda C. M., Armstrong M. *II Biophys. J.*- 1998,-75.-P. 1828-1835.
20. Grishchenko O. V., Kharkyanen V. N., Kononenko N. L., Veinreb G. E. *II J. Biol. Phys.*- 1997.- 23.- P. 195-208.
21. Goushcha A. O., Kliarkyanen V. N., Scott G. W., Holzwarth A. R. *II Biophys. J.*-2000.-79.-P. 1237-1252.
22. Brooks B. L. III, Karplus M., Pettitt M. Proteins. A theoretical perspective of dynamics, structure and thermodynamics // *Adv. Chem. Phys.*-1988.-V. 71.

С. Єсилевський, В. Харк'янен

ЗАГАЛЬНА ТЕОРІЯ ІОННИХ КАНАЛІВ З БАГАТОКРАТНОЮ ЗАСЕЛЕНІСТЮ

У статті представлений загальний теоретичний підхід, що дозволяє описати провідність іонних каналів з множинною заселеністю. Він базується на реальній структурі калієвого каналу KcsA, але не обмежується даним каналом. Показано, що рух іонів у селективному фільтрі каналу є істотно колективним і може бути редукованим до руху єдиної квазічастинки - квазііону. Концепція квазііонів дозволяє пояснити явище безбар'єрної виштовхувальної провідності в селективному фільтрі та уникнути повного опису руху індивідуальних іонів у багатоіонному каналі, що значно спрощує задачу. Показано, що квазііони є фактичними переносниками заряду в каналі.