Hierarchy of Motions and Quasi-Particles in a Simplified Model of Potassium Channel Selectivity Filter

S.O. YESYLEVSKYY and V.N. KHARKYANEN*

Department of Biophysics, Institute of Physics, Natl. Acad. of Sci. of Ukraine, Prospect Nauki, 46, Kiev-03039, Ukraine

(*Author for correspondence, e-mail: vakhar@mail.kar.net)

Abstract. We develop a simplified model of the multiply occupied Kcsa-like selectivity filter based on the best available structural data. The existence of hierarchy of motions in the selectivity filter is shown. Fast fluctuations of the ion-ion distances may be considered adiabatically decoupled from the slow diffusive motion of the ions' center of masses. The latter can be considered as a quasi-particle, called a quasi-ion, moving in an effective potential. In the Kcsa-like selectivity filter occupied by three ions the effective potential allows free barrier-less diffusional motion of the quasi-ions. The concept of the quasi-ions performing ion translocation through the channel may be vital in explaining barrier-less 'knock-on' conduction postulated for real channels.

Key words: hierarchy of motions, Kcsa channel, potassium channel, quasi-particles, selectivity filter

1. Introduction

Theoretical investigation of the ion channels was hampered for a long time by the lack of detailed three-dimensional structure of the channel forming proteins. High-resolution crystal structures of the bacterial Kcsa K^+ channel, first published in 1998 [5], brought new insights into the ion channels science. It was established that the most important feature in the Kcsa structure is a narrow ($\sim 3\text{\AA}$) selective filter. It is responsible for selectivity [1–4], conduction [1–4] and possibly for fast gating of the channels [9–11] and their C-type inactivation [11, 12, 16–18].

The filter has 5 more or less distinct binding sites occupied by K^+ ions or water molecules, however only 3 K^+ ions may be accommodated in the filter due to ion-ion repulsion [1–5, 11]. Recent molecular dynamics (MD) studies revealed barrier-less 'knock-on' conduction mechanism in the filter [2], when the ions move in a concerted manner. An exceptional role of ion-ion forces in the energy balance of different ion configurations on the conduction pathway was established [1–4].

Although selective filter of K^+ channels is recognized long ago to be a multi-ion pore, existing theoretical models fail to describe it adequately. Conventional models of multiply occupied channels usually treat them as a system of binding

sites which may contain only one ion each. Ion-ion interactions are usually treated as restrictions, which allow one ion to reside in each binding site [21]. Such models are inconsistent with the real mechanism of ion translocation in the filter and its estimated energy profile. It is established that there is an extended and deep energy well in the selectivity filter region, which contain several strongly interacting ions [1–4]. The mechanism of conduction in the filter is essentially collective and cannot be described by single-ion jumps.

In the present study we propose a simplified model of the Kcsa-like selective filter based on realistic filter design, energetics and explicit ion-ion interactions. Although we try to make the model as close to the structure as possible, it is not limited to the Kcsa channel. The model is formulated in terms of approximated single-ion energy profiles rather than explicit atomic-resolution potentials, that makes it general enough to describe the whole class of channels with the homologous structure of the selectivity filter. We use the model to investigate the basic physical principles of the ions' motion in the filter. It is shown that the ion translocation may be described by the motion of a quasi-particle, called quasi-ion, through the filter.

2. The Model of the Kcsa-Like Selective Filter

The selective filter of the Kcsa channel may contain up to 3 ions [1–4]. Energy profile, perceived by the single ion in the filter region, is formed by the interaction with the pore walls and with the other ions.

Single ion energy profiles in the filter region were calculated in numerous MD and BD simulations [1–4, 6–8]. Unfortunately, the results are quite different. The majority of methods used in these studies rely on the values of effective dielectric constant of the protein and the pore interior. However, the concept of the effective dielectric constant is hardly applicable to the channel pore which is non-macroscopic and inhomogeneous system. Different effective dielectric constants as well as different strengths of dipoles and values of the wall charges are used in different studies. Thus, it is not surprising that the depth of potential well in the filter region vary from $10.5 k_B T$ [4] to $60 k_B T$ [7]. However, the general shape of the single-ion energy profile is reproducible from study to study.

We use the single-ion potential profile from MD studies of J. Mashl et al [4] as a starting point. This profile may be quite accurately approximated by the inverted Gaussian-like curve:

$$U_0(x) = -a \exp\left(-\frac{x^2}{s^2}\right),$$

where a is the lowest energy in the center of the well, s is the characteristic well width. Comparison with the MD data for the open channel [4] gives $s \approx 9 \text{ \AA}$, $a \approx 10.5 k_B T$ (Figure 1). As it was noted above, the depth of the well is not well established and the work of Mashl et al. [4] actually gives the most shallow

Figure 1. Single-ion energy profile in the selectivity filter for various membrane potentials. The profile for 0 mV is a fit of the experimental profile from [4].

potential well. In the present study we preserve the Gaussian shape of the well and consider two well depths: $a = 10.5 k_B T$ and $a = 60 k_B T$ in order to cover the whole range of the values, obtained in different studies. Our approach is supported by MD simulations performed for various pH values [4] that influence the side-chain effective charges. It is shown that the *shape* of the energy well in the filter is preserved but the maximal well depth can change substantially.

We divide the total electric field in the pore into two parts: the field caused by the membrane potential applied and the field of the other ions in the filter (all the effects of surface charges and interactions with the charged groups and dipoles in the pore wall are assumed to be already included into the single-ion profile). It was shown [1–4] that the membrane potential drops almost linearly in the region of the selective filter in the absence of ions, so the Goldman assumption is valid for the part of the field caused by the membrane potential. The ion-ion interactions are accounted explicitly below. The energy profile perceived by the ion in the presence of the membrane potential is:

$$U_0(x, \phi, a) = -a \exp\left(-\frac{x^2}{s^2}\right) + \frac{q\phi(L-x)}{2L} \cdot \Theta(L-|x|) + q\phi \cdot \Theta(-L-x), \quad (1)$$

where ϕ is the membrane potential, q is the electron charge. $\Theta(x)$ is the Heaviside step function: $\Theta(x) = 0$ for $x < 0$, $\Theta(x) = 1$ otherwise, a is the potential's depth. The membrane potential is a negative constant equal to $q\phi$ inside the cell. It linearly

risks to zero in the filter region and is equal to zero in the external solution as it is adopted in electrophysiology.

Accounting for the ion-ion interactions is also non-trivial. As we already noted, the dielectric constant concept is hardly applicable in the filter of the channel. However, it is obvious that the water molecules and the pore walls lead to some shielding of the Coulomb repulsion between the ions. Thus, the ion-ion interactions may be approximated as a shielded Coulomb repulsion

$$U_{\text{int}}(x_i - x_j, d) = \frac{q^2}{4\pi\epsilon_0} \frac{\exp\left(\frac{-|x_i - x_j|}{d}\right)}{|x_i - x_j|} \quad (2)$$

where ϵ_0 is the vacuum dielectric constant, d is the effective shielding constant that describes the shielding by the pore wall and water molecules situated between potassium ions. It is necessary to emphasize that we use exponential shielding just as a quite reasonable approximation. It should not be treated as Debye shielding nor the constant d should be treated as the Debye factor.

The value of d may be estimated from the assumption of barrier-less conduction as it is described below.

3. Estimating the Shielding Constant

According to the assumption of the barrier-less ‘knock-on’ conduction, the process of ion translocation through the channel does not require surmounting of substantial energy barriers. MD simulations show that the most probable conduction pathway is the following. An ion enters the filter already containing two ions and the state with triple occupancy forms. The ion from the opposite side of the channel is eventually pushed toward the edge and escapes, returning the system to the double occupancy state [2, 3]. All ion configurations on this pathway should have almost identical energies to make the whole process barrier-less. Particularly, it means that the equilibrium states of double and triple occupancies should have identical energies.

Let us calculate the equilibrium ion positions and the energy E_2 of the equilibrium state with two ions inside the filter. It is clear that the ions are situated symmetrically around the energy minimum when the membrane potential is zero, so the energy of the system is

$$E_2(x_2, d, a) = 2U_0(x_2, 0, a) + U_{\text{int}}(2x_2, d), \quad (3)$$

where x_2 is the distance from the center of the well to each of the ions. The same considerations for the state with three ions inside the filter lead to the expression

$$E_3(x_3, d, a) = 2U_0(x_3, 0, a) + U_0(0, 0, a) + U_{\text{int}}(2x_3, d) + 2U_{\text{int}}(x_3, d), \quad (4)$$

where $\pm x_3$ are the equilibrium positions of two ions, while the third ion is situated in the center of the well.

Figure 2. Optimal shielding constant d as a function of the well depth.

Conduction through the filter may be considered barrier-less if the energies of the states with double and triple occupancies are identical

$$E_2(x_2, d, a) = E_3(x_3, d, a). \quad (5)$$

Equality (3) is satisfied only for certain combinations of the well depth a and the shielding constant d , which can be easily calculated numerically. Figure 2 shows the dependence d vs. a . For each well depth there is a certain optimal value of d which allows barrier-less conduction in the selectivity filter. This optimal value increases almost linearly with the increase of the well depth.

For the values $a = 10.5 k_B T$ and $a = 60 k_B T$ used below, the corresponding shielding constants are $d = 2.8 \text{ \AA}$ and $d = 5.6 \text{ \AA}$.

4. Fast and Slow Motions in the Selective Filter

The motion of ions in the selective filter is controlled by two forces of different origin (not counting the random force caused by thermal fluctuations). The first one is the ‘drift force’ caused by the interaction with the pore wall $F_{wall}(x) = \frac{\partial U_0(x)}{\partial x}$ which depends on the ion position on the channel axis. The second is the ion-ion repulsion force which is equal to $F_{ion}(R, d) = \frac{\partial U_{int}(R, d)}{\partial R}$ for each pair of interacting ions separated by distance R . Let us compare these forces assuming that the membrane potential is zero and the channel contains only two ions.

Force F_{wall} reaches the maximal value of $F_{max}(a) = \left| \frac{a\sqrt{2}}{s} e^{-1/2} \right|$ at the points $x = \pm s/\sqrt{2}$ which are bending points of the energy profile (2).

The ion-ion repulsion grows infinitely with the decrease of the distance between the ions. From the equality

$$F_{ion}(R_{cr}, d) = F_{max}(a) \quad (6)$$

we can find the ‘critical distance’ R_{cr} where the ion-ion repulsion becomes as strong as the maximal drift force. For $R < R_{cr}$ the ion-ion repulsion is stronger than the drift force acting on each of the interacting ions regardless of their exact positions. The ion-ion forces grow steeply as $\frac{e^{-R}}{R}$ with the decrease of the distance between the ions, so it is possible to assume that

$$|F_{ion}(R)| \gg |F_{wall}(x)| \text{ for } R < R_{cr} \quad (7)$$

at any point x on the channel axis.

Let us consider the case of the ‘shallow’ potential well with $a = 10.5 k_B T$, $d = 2.8 \text{ \AA}$. The equilibrium distance between the ions is easy to obtain from (3). The critical distance is given by (6).

$$R_{eq} = 2x_2 = 8.23 \text{ \AA}, R_{cr} = 9.32 \text{ \AA}.$$

In the case of the ‘deep’ well with $a = 60 k_B T$, $d = 5.6 \text{ \AA}$ we have:

$$R_{eq} = 2x_2 = 6.62 \text{ \AA}, R_{cr} = 7.68 \text{ \AA}.$$

So, in both cases and condition (7) is satisfied.

In the case of triple occupancy we have:

$$\text{For ‘shallow’ well } R_{eq} = x_3 = 9.89 \text{ \AA}, R_{cr} = 9.32 \text{ \AA}.$$

$$\text{For ‘deep’ well } R_{eq} = x_3 = 7.98 \text{ \AA}, R_{cr} = 7.68 \text{ \AA}.$$

In both cases R_{eq} is slightly larger than R_{cr} , however each ion is influenced by two neighbors. This increases effective value of F_{ion} and condition (7) is still satisfied. Condition (7) is also satisfied for small membrane potentials, but becomes invalid for large potentials, because F_{max} increases with the increase of the applied field.

The obtained results lead us to the following conclusion. If the condition of the barrier-less conduction (5) is satisfied, then individual ions move mostly due to ion-ion forces regardless of the occupancy state, while the drift forces are relatively small. Thus, it is possible to make the following assumption: the motion of ions in the selectivity filter may be decomposed to the slow drift motion due to interaction with the pore walls and the fast vibrations of individual ions caused by strong ion-ion forces. Fast fluctuations of the ion-ion distances are averaged on the time scale of slower drift motion, which may be considered as concerted relocations of all the ions in the filter. This point of view is supported by the experimentally observed ‘knock-on’ conduction performed by the concerted motion of all ions in the filter. It is possible to say that the conduction is performed not by individual ions but by a *quasi-ion* that is a temporary set of ions in the filter, which perform concerted drift motion.

5. Quasi-Ions

Let us consider the *quasi-ion* as a kind of quasi-particle located in the center of masses of ions in the filter. According to the stated above, the motion of quasi-ion is slow in comparison with the fast vibrations of individual ions and can be considered in the adiabatic approximation.

It is convenient to introduce new variables

$$q = \frac{\sum_{i=1}^N x_i}{n};$$

$$p_i = x_i - x_{i+1}, i = 1, 2, \dots, n-1 \quad (8)$$

instead of ion coordinates x_i . q is the position of quasi-ion, 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 terms of new coordinates as

$$x_i = \frac{1}{N} \left(qN + \sum_{k=1}^{i-1} (k \cdot p_k) - \sum_{k=1}^{N-i} (k \cdot p_{N-k}) \right). \quad (9)$$

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

$$r_{ij} = x_i - x_j = \sum_{k=i}^{j-1} p_k. \quad (10)$$

Below we consider the occupancy states with $N \leq 3$. Higher occupancy states are unlikely to be observed with the chosen model parameters due to their very high energies. Allowing for higher occupancy states is trivial and can be easily performed.

The total energy of ions in the channel in each particular moment is

$$E_n(q, \vec{p}_n) = \sum_{i=1}^N U_0(x_i(q, \vec{p}_n)) + \sum_{j>i}^N U_{\text{int}}(r_{ij}(\vec{p}_n)), \quad (11)$$

where $\vec{p}_N$ is a shortcut for $\{p_1, p_2, \dots, p_{N-1}\}$, N is the number of ions in the channel. If $N = 1$ there are no p variables at all.

Let us first consider a ‘closed’ channel which can not exchange the ions with external solutions (the term ‘closed’ is used here to indicate an isolated system detached from external solutions by infinitely high ‘walls’, not a sterically closed channel). The system is in equilibrium and contains N ions. According to (7), the distances between the ions p_i fluctuate much faster than the position of the

ions' center of masses q . Using the adiabatic approximation we can construct the partition function as a function of q :

$$Z_n(q) = \int \exp(-E_n(q, \vec{p}_n)) \cdot d\vec{p}_n. \quad (12)$$

The equilibrium distribution of the quasi-ions along the channel axis is

$$P_n(q) = \frac{Z_n(q)}{\int Z_n(q) \cdot dq}. \quad (13)$$

We can also introduce an effective potential for the quasi-ion as

$$U_n^{eff}(q) = -\ln \left(\frac{Z_n(q)}{\int Z_n(q) \cdot dq} \right). \quad (14)$$

Effective potentials are different for different occupancy states. The shape of the effective potential allows us to determine the character of the quasi-ion motion. In particular, if the bottom of the potential is flat then the quasi-ion can be considered free in that region. This means that the translocation of the quasi-ion requires virtually no energy and is essentially diffusive.

6. Brownian Dynamics Study

In order to have a reference point for theoretical development and for better understanding of the events in the channel we have performed simple one-dimensional Brownian dynamics (BD) simulations of our system. The motion of each ion is restricted to the channel axis and is described by the Langevin equation

$$m\ddot{x} + m\gamma\dot{x} = \frac{\partial E(x)}{\partial x} + F_R(t),$$

where m is the mass of the ion, F_R is the stochastic force caused by thermal fluctuations of the ion's environment, γ is the friction coefficient, E is the potential of all forces acting on the given ion

$$E_n(\vec{x}_n) = \sum_{i=1}^N U_0(x_i) + \sum_{j>i}^N U_{\text{int}}(x_j - x_i). \quad (15)$$

The motion of ions in the channel can be considered as overdamped [2–4], so that

$$\dot{x} = -\frac{1}{m\gamma} \frac{\partial E(x)}{\partial x} + F_R(t).$$

The friction coefficient can be calculated from the ion diffusion coefficient as $\gamma = k_B T / mD$. Using the simplest assumption that the stochastic force is a delta-correlated white noise we have

$$\Delta x = -\frac{D}{k_B T} \left(\frac{\partial E}{\partial x} \right) \Delta t + \sqrt{2D \cdot \Delta t} \cdot \xi(t), \quad (16)$$

where Δx is the change of ion position during short time Δt , $\xi(t)$ is a random variable having normal distribution and unit dispersion.

Equation (16) was used until the ion was inside the channel $-L \leq x_i \leq L$, where L is the half-length of the selectivity filter. In our model $L = 20\text{\AA}$. If the next simulation step drives the ion outside the channel then the ion is instantly removed from the system. There is a certain probability $P(C_i)$ of ion injection into the channel interior from the i -th solution during a single step of simulation. It depends on the effective ion concentration C_i in the corresponding solution. Being injected, the ion is presumed to ‘appear’ in the point $-L$ or L (depending on the injection side) and have velocity $v = \sqrt{2D\xi}$ chosen randomly from the normal distribution.

The current through the channel is calculated as

$$I = \frac{e(N_L N_R)}{t},$$

where e is the charge of the ion (the electron charge), N_L is the total number of ions which have crossed a certain (say, left) boundary of the channel from left to right, N_R is the number of crossings from right to left, t is the total simulation time.

The system is very simple, so it was possible to monitor thousands of ion crossings during the time periods of hundreds of microseconds with the time step of 0.2 ps.

The choice of diffusion coefficient in such a simulation is a non-trivial problem. Different values are used in different studies. It is believed that in the case of one-dimensional motion the diffusion coefficient differs from that in bulk solution. We used the value $D = 0.653 \times 10^{-9} \text{ m}^2/\text{s}$ [6] which is 1/3 of the value in the bulk solution for K^+ ions. The exact value of D is not critical for our simulations because the current is controlled by empirical injection probabilities and the equilibrium distributions of the ions are insensitive to D .

Our primary goal was to determine the value of empirical injection probabilities $P(C_i)$ in order to get the ion currents which are comparable with experimental values. Experimental IV curves of KcsA channel [11] show that the current through the fully open channel is approximately 15 pA at -100 mV . We performed several BD simulations for the membrane potential $\phi = -100 \text{ mV}$, time step $\Delta t = 0.2 \text{ ps}$ and various injection probabilities. In the case of the ‘shallow’ ($10.5 k_B T$) well injection probabilities $P(C_1) = P(C_2) = 0.0001$ correspond to the experimentally observed current of 15 pA. In the case of the ‘deep’ well this current is reached with injection probabilities $P(C_1) = P(C_2) = 0.0002$.

7. Distribution of the Quasi-Ions

The distributions of the quasi-ions along the channel can be calculated from BD simulations as probabilities of finding the quasi-ion in narrow ‘slices’ along the channel axis. The probabilities were monitored separately for each occupancy state

Figure 3. Distribution of the quasi-ions along the channel axis for the membrane potential indicated for the 'closed' and 'open' channels. The depth of the single-ion energy profile $k_B T$.

and then normalized to get a histogram which approximates the distribution function of the quasi-ion $P_i^{(n)}$, where i is the slice number. The following four distinct cases are considered in the present work:

1. 'Closed' channel, $\phi = 0$ mV.
2. 'Closed' channel, $\phi = -100$ mV.
3. 'Open' channel, $\phi = 0$ mV.
4. 'Open' channel, $\phi = -100$ mV.

The distributions obtained are shown in Figure 3 (for the shallow well $10.5 k_B T$) and Figure 4 (for the deep well $60 k_B T$).

Distributions for the 'closed' channel are very sensitive to the membrane potential applied and become very asymmetric for non-zero potentials. This is especially applicable to the distribution for the triple occupancy. This result is quite expectable. Individual ions tend to drift 'downhill' in the potential profile and become cluttered near the left edge of the channel for negative membrane potentials. The ion exchange is prohibited, so that the ions cannot escape and an asymmetric distribution is maintained. In the case of the deep well only the distribution for the triple occupancy is changed considerably. This is explained by the following fact. In the states of single and double occupancy the ions' equilibrium positions are located near the bottom of the potential well. The changes of the energy profile introduced by the membrane potential applied are minimal in this region. In the

Figure 4. Distribution of the quasi-ions along the channel axis for the membrane potential indicated for the 'closed' and 'open' channels. The depth of the single-ion energy profile $k_B T$.

case of triple occupancy the ions are much closer to the edges of the channel where the influence of the membrane potential is much stronger. Distributions which are identical to the 'closed' channel distributions can be obtained theoretically using Eq. (10) (data not shown). This is a direct evidence of the fact that the motion of ions in the filter can be adequately described as that of the quasi-ions.

Distribution for the 'open' channel are, in contrast, much less sensitive to the changes of the membrane potential. Concentration of the probability density on the left side of the potential well is much less pronounced and the overall shapes of the distributions are maintained. So, why the distribution of the quasi-ion in the open channel is so insensitive to the membrane potential applied?

This can be qualitatively explained in the following manner. If the substantial negative membrane potential is applied, the ions will constantly enter the channel from the right, drift through it and exit to the left. However, after entering the channel the distribution of ion-ion distances rapidly relax to equilibrium and actually not individual ions but a quasi-ion drifts through the channel. At each given position on the channel axis the energy of the quasi-ion will not be much different from the equilibrium value of zero membrane potential because of the two factors: 1) the ion-ion distances are still in equilibrium; 2) the shape of the single-ion energy profile near the center of potential well (where the quasi-ion actually exists) is almost independent on the membrane potential. When the leftmost ion

Figure 5. Effective potentials for the quasi-ions for zero membrane potential and the depth of the single-ion potential $a = 10.5 k_B T$ obtained theoretically using (11) and in BD simulations of the ‘open’ channel. The potentials for single, double and triple occupancies are presented.

reaches the edge of the channel, it escapes to solution preventing the quasi-ions to concentrate near the left edge of the channel. In contrast, if the channel is ‘closed’ the quasi-ions will concentrate in that region leading to a strongly asymmetrical distribution. So, the distribution of the quasi-ions is only slightly dependent on the membrane potential applied because the ‘excessive’ density of the quasi-ions on the left is constantly reduced by ion escape and the density ‘deficiency’ on the right is filled with entering ions.

8. Effective Potentials for the Quasi-Ions

In the case of zero membrane potential the whole system is in equilibrium, so that the concept of the effective potential is valid. The effective potential may be obtained theoretically from formulae (11) and from BD data as

$$U_n^{eff}(q_i) = -\ln \left(\frac{P_i^{(n)}}{\sum_i P_i^{(n)}} \right), \quad (17)$$

where q_i is the center of the i -th discrete slice.

Figure 6. Effective potentials for the quasi-ions for zero membrane potential and the depth of the single-ion potential $a = 60 k_B T$ obtained theoretically using (11) and in BD simulations of the ‘open’ channel. The potentials for single, double and triple occupancies are presented. See the text for the explanation of the large deviations of single and double occupancy potentials from the theoretical curves.

Figures 5 and 6 show the theoretical effective potentials for the ‘closed’ channel in equilibrium and the results of BD simulations of the ‘open’ channels in the case of zero membrane potential and shallow ($10.5 k_B T$) and deep ($60 k_B T$) potential wells respectively. It is clearly seen that BD simulations of the ‘open’ channel give the effective potentials which are quite similar to theoretical. Within the region $\approx \pm 5 \text{Å}$ around the center of the filter they are essentially identical. This correspondence is a convincing proof of the quasi-ions’ existence in our model. Therefore, the underlying assumption of the hierarchy of motions in the selectivity filter and adiabatic decoupling of the slow motion of the center of masses is also proved. It is also evident that the interior of the filter remains approximately in equilibrium even if the ion exchange events are considered explicitly.

Observed deviations from theoretical effective potentials are caused by the fact that the latter are calculated in the assumption of the ‘closed’ channel. The probability of finding a quasi-ion near the edge of the ‘open’ channel is substantially reduced by the escapes of ions to external solution. This feature is especially pronounced in the case of triple occupancy. However, if the quasi-ion is located near

the center of the filter the escape probability is small and effective potential is very similar to theoretical in this region. Large deviations of the effective potentials for single and double occupancies in the case of the deep ($60 k_B T$) well are caused by the following. Let us consider the state of double occupancy. Very large depth of the well makes the probability of finding the ion at the points $|x| > 3\text{\AA}$ very low. However, when the ions are exiting the channel in the triple occupancy state, one of the remaining two ions appears to reside sufficiently far from the center. It relaxes rapidly, moving closer to the center, but the short time spent in the regions $|x| > 3$ is enough to produce the observed artifacts. The same situation is observed in the single occupancy state. In this case deviations are produced by the escape events from the double occupancy state.

The effective potential for the single occupancy coincides with the single-ion potential (2). A single ion should surmount an energy barrier a to escape that is highly improbable even in the case $a = 10.5 k_B T$ and can be considered absolutely impossible in the case $a = 60 k_B T$. Effective potentials become much wider in the double occupancy state. The shapes of the effective potentials for the triple occupancy state are remarkably different. The ‘bottom’ of the effective potential within the region $\approx \pm 5\text{\AA}$ is almost ‘flat’. It means that the motion of the quasi-ion near the center of the channel is almost free diffusion. In reality, if the quasi-ion moves, say, towards the left edge of the channel, the leftmost ion reaches the edge and escapes to solution. However, in the ‘closed’ channel it remains in the channel. This leads to the ‘cluttering’ of ions and to a very steep increase of the effective potential at the edges of the channel.

In terms of quasi-ions the whole conductance pathway through the selectivity filter can be imagined as following. The ion enters the filter already containing two ions. After the rapid initial relaxation the distribution of the ion-ion distances reaches the equilibrium that marks the formation of the quasi-ion in the triple occupancy state. The quasi-ion diffuses through the filter in the nearly flat effective potential. During this process the ion-ion distances fluctuate around their equilibrium values. Finally, one of the ions reaches the edge of the channel and escapes returning the system to the initial state. It is important that the quasi-ion may not leave the ‘flat’ region of the effective potential near the center of the filter to perform conduction. Fluctuations of the ion-ion distances that do not change the position of the center of masses too much, still may push an individual ion to the edge of the channel. Thus, the conduction remains essentially barrier-less.

9. Conclusions

In the present work we introduce a simplified model of the selectivity filter of the potassium ion channel with multiple occupancy. The model incorporates the results of recent structural studies and molecular dynamics simulations to provide a realistic description of the channel structure and conductance mechanism. It is shown that there is a hierarchy of motions in the selectivity filter which is described

by a wide energy well with multiple occupancy. The fastest motions are fluctuations of the ion-ion distances, which can be considered in equilibrium regardless of the membrane potential applied. The second level of hierarchy is the motion of the ions center of masses, which can be viewed as a quasi-particle called quasi-ion. This motion is much slower and may be considered adiabatically decoupled from fluctuations the ion-ion distances. We found that these results are observed for very different depths of the energy well in the filter region. This makes our results consistent with a wide range of MD and BD studies which give different depths of the single-ion energy profile. The only assumption, which allows to consider the motion of ions in the filter as a translocation of the quasi-ions, is the assumption of the barrier-less conduction supported by the recent studies.

The quasi-ions move in the effective potential, which depends on the channel occupancy. The effective potential for triple occupancy in the selectivity filter is nearly 'flat' allowing free, barrier-less diffusion of the quasi-ions. We have found that the distribution of the quasi-ions along the channel axis is only slightly dependent on the membrane potential applied. Quasi-ions are constantly regenerated by the incoming ions at one side of the channel and break down at the other side where the ions exit the channel. Conduction through the channel can be viewed as an almost free diffusive motion of the quasi-ion intervened by the entering and escapes of individual ions. These results can be vital to explain barrier-less 'knock-on' conduction mechanism observed in molecular dynamics studies.