Is the mobility of the pore walls and water molecules in the selectivity filter of KcsA channel functionally important?

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We performed in-depth analysis of the forces which act on the K^+ ions in the selectivity filter of the KcsA channel in order to estimate the relative importance of static and dynamic influence of the filter wall and water molecules on ion permeation and selectivity. The forces were computed using the trajectories of all-atom molecular dynamics simulations. It is shown that the dynamics of the selectivity filter contributes about 3% to the net force acting on the ions and can be neglected in the studies focused on the macroscopic properties of the channel, such as the current. Among the filter atoms, only the pore-forming carbonyl groups can be considered as dynamic in the studies of microscopic events of conduction, while the dynamic effects from all other atoms are negligible. We also show that the dynamics of the water molecules in the filter can not be neglected. The fluctuating forces from the water molecules can be as strong as net forces from the pore walls and can effectively drive the ions through the local energy barriers in the filter.

Introduction

Ion channels are transmembrane proteins that form highly selective aqueous pores in the lipid bilayer. They allow the ions crossing the hydrophobic core of the membrane, facilitating the control of ionic concentrations in the cell.[1,2] Ion channels differ in functional, gating and selectivity properties.[3–7] All the K^+ channels (including voltage-gated K^+ channels[8,9]) share the same core structure. Tetrameric K^+ channels, like KcsA, are probably the most widely studied class of the ion channels because of their functional importance and the availability of several high-resolution crystal structures.[10–14] The structure of the KcsA K^+ channel is shown in Fig. 1. Each subunit contains two transmembrane helices (M1 and M2) with an intervening P-loop. The central structural element of the KcsA, conserved in all K^+ channels, is the selectivity filter. It is located close to the outer mouth of the channel and forms the narrowest part of the pore. The filter extends over about 12 Å with a mean radius of about 1.4 Å. It exhibits highly conserved TVGYG sequence and is responsible for the selective permeation of potassium ions.[12] The backbone carbonyl groups of the filter residues form five binding sites (S0–S4) that can be occupied by either K^+ ions or water molecules. Two additional sites (S_{EXT} and S_{CAV}) were elucidated experimentally at the extra and intracellular mouths of the filter, respectively. Under physiological conditions two or three K^+ ions occupy the filter. They reside in non-adjacent binding sites with the water molecules intercalated between them.

Computational studies, such as Molecular Dynamics (MD), Brownian dynamics and the quantum chemical calculations, are currently the only techniques which allow study of the mechanism of conduction and selectivity in the potassium channels in atomic detail.[15–26] The role of the selectivity filter mobility in permeation is argued since the first attempts of modeling the potassium KcsA channel. There is a hypothesis, widely accepted by the community, that the filter wall plays an important role in the selectivity and conductance of the channel.[27] This hypothesis is supported by the observation of concerted motion of the ions and the carbonyl groups, which form the filter wall.[28] It was also shown that the conformation of the filter wall depends on the positions of K^+ ions in the filter.[27] Recently, an induced-fit mechanism was proposed as explanation of selectivity.[28] This concept takes into account the dynamics of the hydration processes in the filter. The model of freely fluctuating carbonyls, which was proposed recently, shows significant correlation between
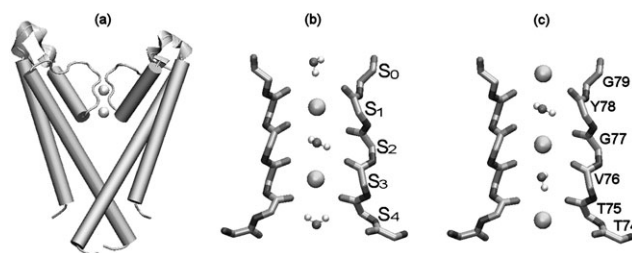

Fig. 1 Schematic representation of the KcsA channel structure. Only two of the four subunits are shown for clarity. (a) The whole channel as it appears in the crystal structure. (b) The selectivity filter with the double occupancy (S1,S3) configuration. Positions of the (S0–S4) binding sites are indicated. (c) Selectivity filter with the triple occupancy (S0,S2,S4) configuration. The residues forming the TVGYG sequence are also indicated.

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the mobility of the structure and some microscopic events at the filter. However, all these data are obtained in rather short MD simulations (nanosecond time scale), which do not allow to estimate the current through the channel (microsecond time scale). As a result it is not known if the filter dynamics influences the macroscopic characteristics of the channel, such as the current or permeability ratio for different ions.

In this work we performed detailed analysis of the electrostatic and the van der Waals (VDW) forces, which act on the ions located in the selectivity filter of KcsA channel using the results of all-atom MD simulations. The static and dynamic influences of the filter wall and water molecules, located in the filter, on the permeating K^+ ions were studied. Our results allows to clarify the role of the flexibility of the filter wall in permeation and shows an important role of water molecules in driving the ions through the binding sites in selectivity filter.

Methods

The molecular dynamics simulations

The model of the KcsA channel was built using the experimental X-ray structure with 2.0 Å resolution (PDB code 1K4C[12]). The lipid bilayer was modelled by an octane slab, which mimics the hydrophobic core of the membrane.[30] Two slabs of water molecules were added from both sides to solvate the hydrophilic parts of the protein. The thickness of the octane slab is approximately 29 Å. The protein is positioned in such a way, that the residues Trp66 and Trp92 of each subunit are located close to two octane–water interfaces. The protein's cavity was hydrated with approximately 40 water molecules, which corresponds to the density determined by evaluating the Connolly surface.[31] The cases of double and triple ionic occupancies were studied. In agreement with Zhou et al.[12] the binding sites were filled by two distinct alternate sequences of K^+ ions and water molecules, namely WKWKW and KWKWK as shown in Fig. 1b and c. Our sequences of K^+ ions and water molecules in the filter will be referred according to the positions of the ions as (S1,S3) and (S0,S2,S4) sequences, respectively.

All MD calculations were carried out using the AMBER suite of programs with the AMBER8 force fields.[32] The Coulomb interactions were treated using the particle mesh Ewald (PME) method.[33] Water molecules were treated using the TIP3P model.[34] In order to be consistent with our previous simulations, the polarization interactions were switched off.[29] The integration step of 1 fs was used in order to account for all motions including the fastest water rotations. The protein was first equilibrated with two ions in the binding sites 1 and 3 ((S1,S3) sequence) during 3 ns. Then the simulation was stopped, three ions were placed into binding sites 0, 2 and 4 ((S0,S2,S4) sequence) and the whole system was equilibrated again for 3 ns. Production runs of 3 ns were performed for the systems with double and triple occupancies in NPT conditions. The trajectories were recorded with 1 ps intervals. The constant temperature was maintained with the Berendsen weak coupling algorithm.[36] The details of the simulation protocol are described in our previous papers.[29,35]

Force calculations

In order to estimate the influence of the dynamics of the filter walls on the permeating ions we evaluated the electrostatic and the VDW forces, which would act on the ion located in different positions of the channel axis. The force component, directed along the channel axis, was evaluated with small (0.1 Å) discrete steps and averaged over all trajectory frames. Several major assumptions were introduced:

(1) Possible radial motions of the ions are excluded from consideration. It is assumed that these motions are fast and can be averaged for each axial position of the ion.[37]

(2) No explicit interaction with water molecules is considered at this stage (the influence of water is analyzed separately below).

(3) The coordinates of the filter atoms are obtained in the conditions where the ions occupy one of the two stable configurations, namely (S1,S3) and (S0,S2,S4), while the forces are evaluated in all possible positions along the channel axis. As a results alternative configurations of the filter wall, which correspond to other placement of ions in the translocation process are not sampled. This is an inevitable consequence of restricted simulation time and the limited sampling.

The total non-bonded force F, which acts on the ion from particular atom i at particular trajectory frame k is

$$F_{ik}(z) = \left[a \left(\frac{6c_{i6}}{r_{ik}(z)^7} - \frac{12c_{i12}}{r_{ik}(z)^{13}} \right) + b \frac{q_{ion}q_i}{r_{ik}(z)^3} \right](z_{ik} - z)$$

where z is the position of the ion along the pore axis; c_{i6} and c_{i12} are the coefficients of the VDW interaction between K^+ and the ith atom defined in the force field; q_{ion} is the ion charge, q_i is the partial charge of the ith atom; $r_{ik}(z)$ is the distance between the ion and the ith atom for given frame; z_{ik} is the z coordinate of the ith atom for a given frame; a is the coefficient which converts the VDW energy into k_BT units if c_6 and c_{12} coefficients are expressed in kcal mol^{-1} Å^{-6} and kcal mol^{-1} Å^{-12}, respectively and the distance is in angstroms; b is the coefficient, which converts the electrostatic energy into k_BT units if the charge is expressed in elementary charges and the distance is in angstroms. For $T = 300$ K, $a = 1.6788$, $b = 566.2$.

We calculated the mean force acting on the ion in each position on the channel axis as

$$F_{mean}(z) = \frac{1}{N_{fr}} \sum_{k=1}^{N_{fr}} \sum_{i=1}^{N} F_{ik}(z)$$

where N is the number of atoms in the subset under study, N_{fr} is the number of trajectory frames.

The single-ion energy profile is calculated as:

$$E_{ion}(z) = - \int_{z_{min}}^{z_{max}} F_{mean}(z) dz$$

where z_{min} and z_{max} are the boundaries of the filter in z direction measured from the center of masses of all heavy atoms in the filter. We assume that $z_{min} = -20$, $z_{max} = 20$, which is consistent with the conventions adopted in ref. 38.
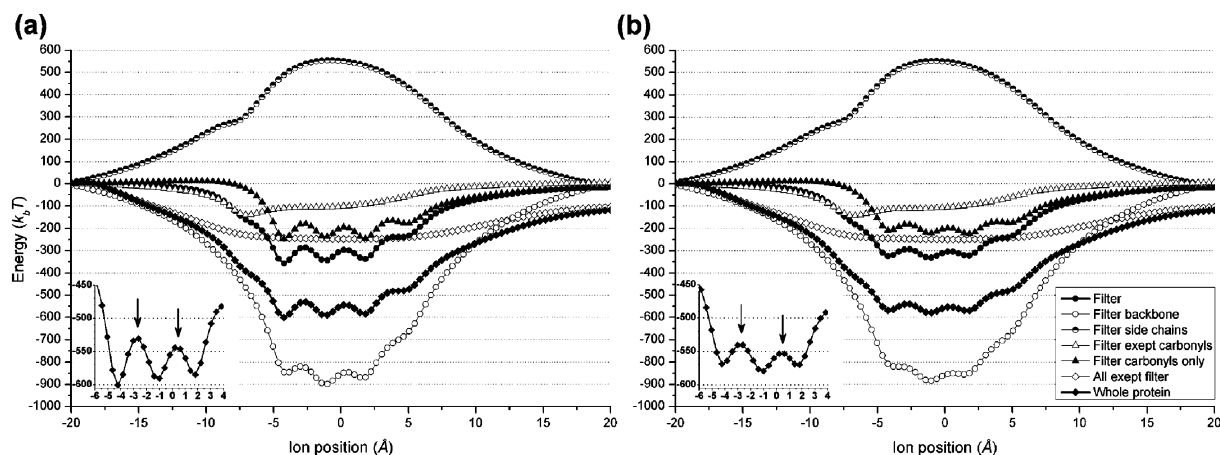
Fig. 2 Single-ion energy profiles in the selectivity filter region of KcsA channel produced by various subsets of atoms in presence of 2 (a) and 3 (b) ions. The insets show the energy barriers between the binding sites, which are indicated by the arrows. On this and all subsequent figures the Z coordinate of the center of masses of all heavy atoms of the filter-forming TVGYG residues is assumed to be zero.

The root means square (RMS) fluctuations of the mean force are computed as:

$$\Delta F_{\mathrm{rms}}(z) = \sqrt{\frac{1}{N_{fr}} \sum_{k=1}^{N_{fr}} \sum_{i=1}^{N} (F_{ik}(z) - F_{\mathrm{mean}}(z))^2}$$

The contribution of particular atom i into the RMS fluctuation of the force is computed as:

$$\Delta F_i(z) = \sqrt{\frac{1}{N_{fr}} \sum_{k=1}^{N_{fr}} (F_{ik}(z) - F_{\mathrm{mean}}(z))^2}$$

All force calculations were performed in VMD 1.8.6[39] using custom TCL scripts.

Results and discussion

Single ion energy profile in the filter region

Single-ion energy profiles play an important role in understanding the behavior of the ion channels. The single-ion energy profile is defined as the free energy of the single ion placed into a given position of the channel axis. Later on we will use the term "energy profile" instead of the "free energy profile" for the sake of simplicity. Single-ion energy profiles in the selectivity filter of KcsA channel were calculated in several studies.[38,40] It was shown that the profile has an inverted bell-like shape with a relatively small local minima at the bottom, which corresponds to experimentally determined binding sites for the K^+ ions and the water molecules. While the shape of the profile can be considered well-established, its depth is still uncertain. The values found in the literature vary from 10 to 200 kBT depending on the calculation technique and the assumptions made.[41] In the present work we calculated the single-ion energy profile of electrostatic and VDW forces between the channel wall and the ion in the pore. We computed the profile created by the whole channel-forming protein and extracted the parts of this profile, created by various functionally important subsets of atoms in the structure, such as the whole filter, filter backbone, filter carbonyls *etc*. The

results presented in Fig. 2 correspond to the cases of double and triple occupancies of the selectivity filter.

It is well seen that the fine shape of the energy profile is created mostly by the carbonyl groups of the selectivity filter, while the other atoms of the filter backbone increase the overall depth of the potential. It is interesting to note that the influence of the filter side chains is repulsive, while the influence of the filter backbone is attractive to the ion. The rest of the protein imposes a very strong effect on the single ion energy profile in the filter despite the large distance from the ions. The filter itself produces the energy well of ~300 kBT, while the whole protein make this number twice larger—around 600 kBT. This means that the remote regions of the channel protein have a strong influence on the ions in the filter and their contribution cannot be neglected in the quantitative studies. At the same time the shape of the energy profile is not changed by these long-range interactions. Moreover, different ion sequences in the selectivity filter do not change the shape of the profile significantly. The only detectable difference between the cases of double and triple occupancies is the heights of the energy barriers between the binding sites. These barriers are approximately two times larger in the case of double occupancy (insets in Fig. 2). This observation confirms the well-known fact that the (S0,S2,S4) sequence is more stable than the (S1,S3) one.[25] It is not completely clear, however, what causes this difference. To address this question we computed average structures of the whole channel for the trajectories, which correspond to the double and triple occupancies of the selectivity filter and compared them. The root mean square deviation (RMSD) between these aligned structures is 0.58 Å. The RMSD deviation of the aligned selectivity filter regions of the average structures is even smaller (0.192 Å). This means that the average structure of the channel is almost independent on the sequence of the ions in the selectivity filter in our simulations. We did not study in details how observed minor structural differences can cause quite significant changes of the barrier heights, which are visible in Fig. 2. The most probable reason is the very small distance between the filter carbonyl groups and the ions in the binding sites, which leads
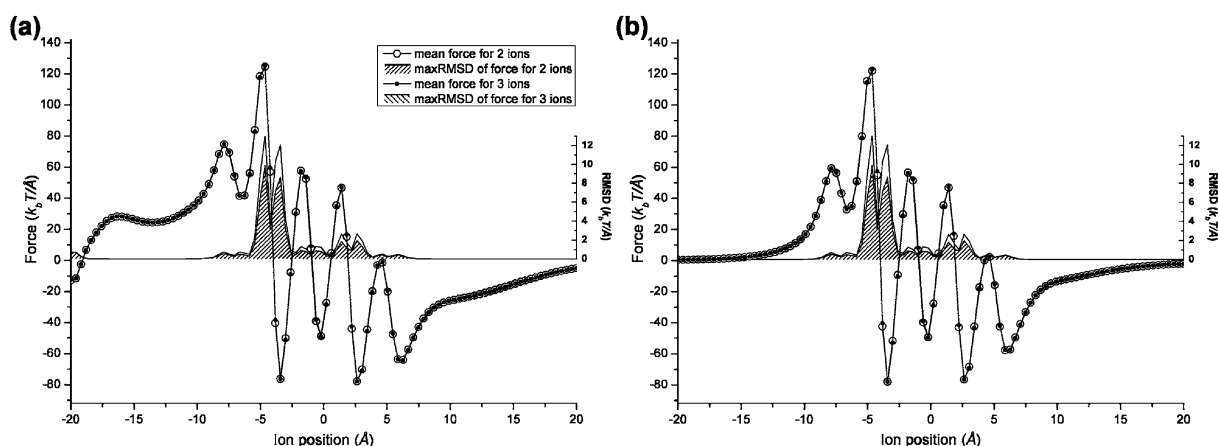
Fig. 3 Mean force and its associated RMSD fluctuations acting on the ion from all protein atoms (a) and from the selectivity filter only (b) in the presence of two or three ions in the filter as a function of ion position along the pore axis. Note the different scales used for forces and their RMSDs.

to extremely steep dependence of the energy profile in this region on the positions of the carbonyls.

We also computed the fluctuations of the filter structure during the simulations with particular occupancy. These fluctuations are much larger than the RMSD between the average structures for different occupancies (~ 0.5 Å and 0.192 Å, respectively). It is possible to speculate that during the simulations the instantaneous structure of the filter samples approximately the same conformational space for both occupancies, but the probabilities of different configurations depend on the occupancy, which results in slightly different average structures. This assumption should be verified by additional simulations, however.

In general the single-ion energy profile and the average structure of the channel are surprisingly insensitive to the occupancy of the selectivity filter in our simulations. We are aware that the simulation time is quite small and thus only small part of accessible conformational space is sampled. However, our data allows concluding that the general shape of single-ion energy profile in the filter region is insensitive to the occupancy of the filter, while the heights of the energy barriers between the binding sites can change significantly upon change of occupancy.

It should be emphasized that the electrostatic forces were computed in the present work by summing up the Coulombic terms for the protein atoms, while the MD trajectories were obtained using the PME summation of electrostatic forces over the multiple periodic images of the system.[33] This means that the calculated absolute depth of the single ion energy profile can be somewhat different from the real value observed in MD calculations. This can not, however, change the results and conclusions qualitatively.

The role of dynamics of the filter walls

The single ion energy profile is the time-averaged quantity. It is created by the mean force acting from the fluctuating channel structure to the ion in the filter. This force can be decomposed into the static component created by the static structure, "frozen" in the average conformation, and the fluctuating component, created by the fluctuations of the channel structure around this average conformation. To our knowledge the magnitude of this fluctuating force component was not studied in details. To estimate the magnitude of the force fluctuations, we calculated the RMSD of the force acting to the ion in each point on the filter axis. The results are shown in Fig. 3.

It is clearly seen that the magnitude of force fluctuations is rather small in comparison to the average force itself (note different scales in Fig. 3). RMSD of the force changes in accord with the force itself and reaches the maximum at the maxima of the force (the absolute value of the force is considered). The RMSD constitutes generally only 3% of the average force. This allows concluding that the dynamics of the filter does not change the force acting on the ion significantly and, as a consequence, does not influence the motion of the ions to the extent, which can be detected in most simulations. It is remarkable that the fluctuations of the force are identical for double and triple occupancy. This means that the sequence of the ions and water molecules in the selectivity filter does not influence the dynamic contribution of the selectivity filter walls to the force, which is acting on the ions.

Our data shows that the fluctuations of the filter atoms can be neglected and the filter can be considered essentially static for the majority of purposes, which imply the calculation of the macroscopic characteristics of the channel, such as current. In contrast, in order to study short time events, which are important for understanding channel selectivity, the filter has to be considered highly flexible and the carbonyl groups should be treated explicitly. This approach was also proposed by S. Yu. Noskov and B. Roux in their "toy model" to explain the selectivity of KcsA and NaK channels.[28]

Dynamics of individual atoms in the filter wall

Despite the fact that the dynamics of the filter can change the average force acting on the ions by only 3% in average, it is still possible that the motions of particular atoms in the filter wall influence the selectivity in the filter or other microscopic events of conduction. In order to study such events one can run sufficiently long MD simulations of the whole channel, but this seems to be excessively detailed, since it is obvious that only the atoms in the close proximity to the ion can change its

motion significantly. In principle, it is enough to study the dynamics of only few atoms, which influence the ion directly on the short distance and keep the rest of the structure fixed, since the dynamic effects from the distant atoms are obviously negligible. Despite numerous technical problems, such partitioning was recently used in the molecular dynamics[28] and the Brownian dynamics[42] studies of KcsA channel. However, the subset of atoms, which were considered dynamic in these studies, was chosen rather arbitrarily. In order not to include excessive number of moving atoms into simulation, one has to know which atoms constitute the dynamic subsystem.

In order to clarify this question we computed the RMSD of the force acting on the ion created by each atom of the channel separately. This quantity was then normalized with respect to the net force acting on the ion from the whole filter and finally the maximal values of RMSD along the pore axis were recorded. To our knowledge this is the first attempt analyzing the dynamic influences of each individual atom in the channel structure on the motion of the ions. Fig. 4 shows the results of these calculations. There are eight pronounced peaks of RMSD, each pair of which corresponds to the single subunit of the KcsA channel. The larger peaks correspond to the filter-forming residues. The smaller peaks (with lower RMSD values) correspond to the gate region and more precisely the interaction with the Thr107 residue located in this narrow part of the protein. The fine structure of each large peak is shown in Fig. 4b. Each subunit produces four close peaks, which correspond to four filter-forming residues, namely Thr75, Val76, Gly77 and Tyr78. The last Gly79 of the well-known filter forming sequence TVGYG only produces a small contribution to the net force. There are only two atoms in each residue, which exhibit significant value of RMSD, namely the backbone carbonyl oxygen and the backbone carbonyl carbon, which are located very close to the ion. The fluctuations of the force produced by the motion of these atoms constitute around 3% of the net force acting on the ion. So, it is evident that the overall dynamic contribution of the filter is effectively reduced to the dynamic contribution of only 32 atoms (two atoms in each of four filter residues in each of four subunits)

from the pore-forming carbonyl groups, while the dynamic contribution of all other atoms is insignificant. In principle this opens the possibility to construct very simple and time efficient simulation protocol for studying the microscopic events in the filter, which has only about 100 moving particles (filter carbonyls, ions and water molecules). The "toy model"[28] mentioned above can be used as a basis for such simulation protocols, which are, however, out of the scope of the present study.

The role of water

The role of water molecules in the functioning of the selectivity filter can hardly be overestimated. It is well known that the selectivity itself originates in a difference in dehydration costs for different ions.[12] However, the role of the water molecules in the narrow part of the filter is still a subject of discussion. In the context of the current work it was interesting to evaluate the force which acts on the ion in the filter from the water molecules. Unfortunately, it is not possible to include this force into the single-ion energy profile since the ions are confined by their initial binding sites during the MD simulations and the lengths of available trajectories are too small to sample other positions of K^+ ions. That is why we were only able to calculate the force, which acts on the ions fluctuating around their local equilibrium positions. This samples only the close proximity of the initial binding sites, but still provides some insight into the role of water molecules in permeation. We summed up the electrostatic and VDW forces from all water molecules located within the 25 Å from the ion of interest for each frame of the trajectory and plotted them as a function of the ion position. The computations were performed for the two trajectories, namely for the (S_1, S_3) and (S_0, S_2, S_4) configurations (Fig. 5).

In the case of double occupancy (Fig. 5a), the force from the water molecules is significantly different for the inner and outer ion (see average bar). The average force acting on the outer ion, located in the binding site S_1, is almost zero. By contrast, the average force acting on the inner ion, located in the site S_3, is about 12 $k_B T/Å$. The fluctuations of the force are
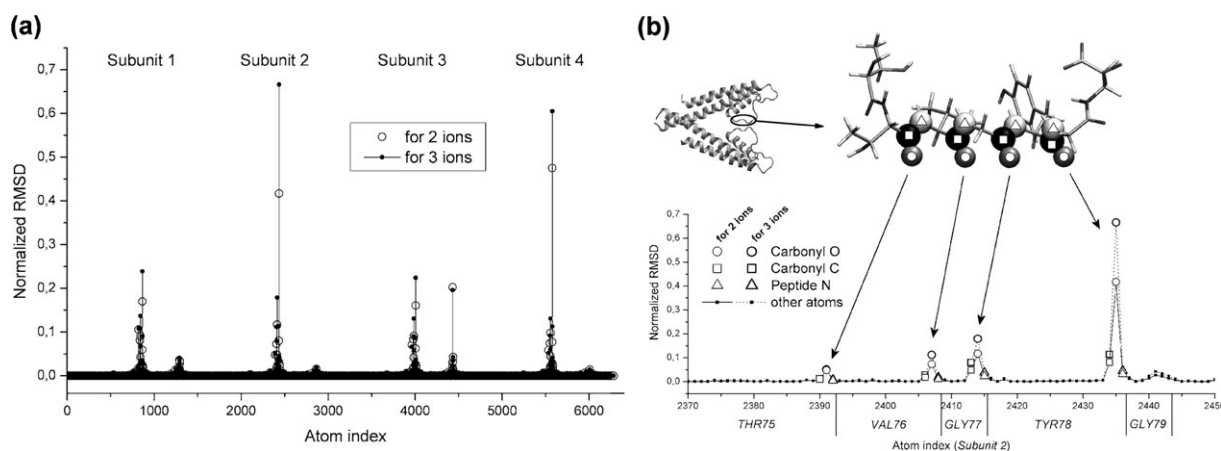

Fig. 4 (a) The maximal RMSD fluctuations of the force acting on the ion at every Z position from individual atoms normalized by the net force acting on the ion. Data collected for all atoms in the channel-forming protein in the presence of 2 and 3 ions in the filter. Each peak corresponds to TVGYG residues in one monomer. (b) Data zoomed on the filter residues (Thr75, Val76, Gly77, Tyr78 and Gly79) of the second monomer corresponding to structure with 2 and 3 ions.
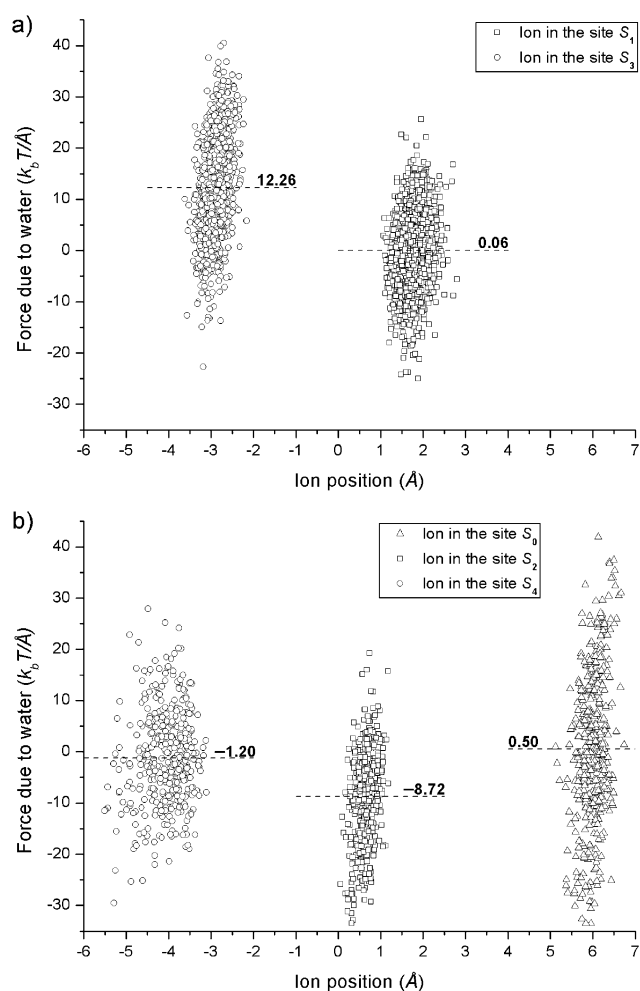
Fig. 5 The force acting on each ion from the water molecules located within 25 Å from the ion: (a) Two ions are located in the binding sites S_1 and S_3. (b) Three ions are located in the binding sites S_0, S_2 and S_4. For clarity, the values of average forces are given and indicated by the dashed lines.

also much larger for the inner ion. In the case of triple occupancy (Fig. 5b), the average forces for the ions in the sites S_0 and S_4 are essentially zero, while the ion in the binding site S_2 exhibits an average force of about $-9\,k_BT/\text{Å}$. However, the magnitude of force fluctuations can reach $40\,k_BT/\text{Å}$ for the outer ion and $30\,k_BT/\text{Å}$ for the inner ion. By contrast, the force fluctuations for the S_2 ion are smaller and reach only 20 $k_BT/\text{Å}$, which reflect the stronger confinement of this ion between the carbonyl rings.

In general, the maximal force acting on the ions from the water molecules constitutes as much as 50–150% of the net force, which acts on the ion at the same positions from the filter walls (as is evident from comparison with Fig. 3). This maximal force is observed only rarely during large fluctuations of the positions and orientations of the water molecules. However, such events can be crucial for conductance since they can allow jumps between the binding sites inside the filter, which are otherwise separated by prohibitively high energy barriers. This allows us to conclude that the dynamics of the water molecules is much more significant then the dynamics of

the filter wall. This also strongly discourages from the usage of implicit solvent models in the modeling of the events in the selectivity filter, since it is clear that continuous models can not mimic the fluctuating forces imposed by the water molecules.

Conclusions

The ultimate goal of the majority of simulations of the ion channels is the prediction of their conductance. In this sense it is very important to know the amount of details, which should be included into the model in order not to lose the accuracy. Our work provides the evidence that the structure of the selectivity filter can be considered static in the simulations focused on obtaining the macroscopic properties of the channel. Although the selectivity filter displays considerable flexibility in our simulations (RMSD of ∼0.5 Å) the force fluctuations, which originate from the structure fluctuations, remain very small. It is shown that the dynamics of the filter during the MD simulations can change the forces, which act on the permeating ions, by only 3% in comparison to the static average structure. Since the accuracy of the conductance calculations is usually smaller than this, accounting for dynamic effects is not justified.

It is shown that the dynamic contribution of the filter is independent on the filter occupancy, while its static contribution can be sensitive to small changes of the filter structure caused by different number of ions in the filter. The RMSD between the average structures of the selectivity filter in the cases of double and triple occupancies is only 0.19 Å in our simulations, however this leads to significant change of the heights of the energy barriers between the binding sites in the filter. Despite this differences the general shape of the single-ion energy profile is insensitive to the occupancy.

Our data suggest that only the carbonyl atoms of the filter forming residues can be considered as moving, while all the other structure of the filter can be modeled as static for the majority of applications. The dynamic influence of the carbonyl atoms can be as large as 3% of the net force acting on the ions, while the dynamic influence of all other atoms is negligible.

Finally, we have found that the dynamic effects of the water molecules are an order of magnitude larger than the dynamic effects of the filter atoms. Instantaneous forces from the water molecules can be so strong, that they effectively counterbalance the net force acting on the ion from the filter wall. Despite the fact that such events are rare, they can play a crucial role in hoping over the energy barriers between the binding sites. Our data suggest that fluctuations of the water molecules in the filter as well as the repulsion between the K^+ ions can lead to the knock-on translocation process. Our data also suggest that implicit solvent models should not be used for simulations of the selectivity filter.

Acknowledgements

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