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Influence of Interionic Interactions on Functional State and Blocker Binding of Voltage-Gated Potassium Channels

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Abstract—A mechanism of ion conduction of a voltage-gated potassium channel KcsA was investigated in full-atomic approximation at a trajectory length of 100 ns using the Lomonosov supercomputer. Methods of molecular dynamics were employed. A structure of the KcsA channel in the open state obtained by X-ray structure analysis (PDB ID 3fb7) was used. Free energy profiles of the KcsA pore occupied with either one or three potassium ions were calculated. It was shown that, under physiological conditions, ions pass through the channel pore cooperatively and the mechanism most probably includes three ions permeating in concert. Interactions of the mammalian voltage-gated channel Kv1.2 with neurotoxin were investigated. It was demonstrated that the effect of interionic interactions on binding of a blocker is rather insufficient.

Keywords: voltage-gated potassium channels, neurotoxin, molecular dynamics, interionic interactions.

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INTRODUCTION

Voltage-gated potassium channels (Kv) represent the most broad and diverse group of ion channels. They play a critical role in functioning of excitable cells and are used as targets in new drug design for various pathologies and many inheritable diseases. Since proteomics of ion channels is of crucial importance, collecting the fundamental data about the structure of ion channels, mechanisms of their function, and their interaction with ligands (blockers) is essential for the development of biomedical applications. At present, molecular dynamics methods [1] that employ supercomputer technologies are widely used to study the dynamics of ion channels and processes of ion transport, including ion conduction values and their dependence on various external factors [2], the dependence of ion free energy on its position along the channel axis [3], structural characteristics and the energetics of different stages of ion permeation through the selectivity filter (SF) of the channel [2, 4], and effects of interionic interactions.

Since ion channels are characterized by a relatively high level of homology, it is expected that the main principles of blocker binding and the mechanisms of ion transport would be rather similar throughout the whole Kv group of ion channels, including both eukaryotic [5] and prokaryotic [6] channels. The specific function of ion channels is to provide a subtle regulation of electrostatic interactions between charged groups of a protein, ions, and water dipoles. In the present work, we use molecular dynamics methods to study the functioning of the bacterial potassium KcsA channel, the role of interionic interactions in the dynamics of ion transport through the channel pore, and the effect of electrostatic forces on binding of the eukaryotic potassium Kv1.2 channel with the blocker (agitoxin) in solutions of different ionic strengths.

MATERIALS AND METHODS

Simulations of Ion Transport Dynamics in Voltage-Gated Potassium Channels

Simulations were carried out using the bacterial KcsA channel as an example. To obtain a molecular dynamics KcsA model, the open state structure of the channel recently obtained by X-ray analysis (PDB ID 3fb7) was used [7]. The structure resolution was 0.33 nm and the pore radius at the channel gate was calculated as 0.5 nm using the HOLE program [8]. The SF and the cavity in front of the SF were saturated with potassium ions and water molecules. In the obtained structure two ions were located at sites S4 and S2, one ion was positioned within the channel cavity (Scav site), and water molecules were located at sites S3 and S1. The simulations of the KcsA channel were conducted using the AMBER03 potential force field [9]. Ionization state of all ionizable amino acid residues of KcsA, except E71, was assumed as pH = 7. The E71 residue was assumed to be in the protonated form. It should be mentioned that the protonated E71 forms hydrogen bonds, which play an important role in the

formation of the channel selectivity filter structure [10].

The model of a phospholipid membrane was built based on the structure of dipalmitoylphosphatidylcholine (DPPC) [3, 10]. The dimensions of the unit cell were $9 \times 9 \times 10$ nm. The membrane simulations were conducted in heavy atom approximation. Force field parameters and torsion angle dynamics equations were used as described in [11]. The protein was docked into the relaxed phospholipid bilayer using the G_MEMBED program (GROMACS software) [12]. Step relaxation of the obtained molecular system was applied. At the first step, the regime of Langevin dynamics with the restrained motion of the protein and ions was realized (program software AMBER [13]). At the initial step of the relaxation process, the protein molecule center-of-mass and potassium ions inside the SF were restrained using a harmonic potential with a force constant of 0.1 kcal/(mol nm^2). The trajectory length of the initial relaxation regime was set at 2 ns as recommended in [12]. The electrostatic interaction energy was calculated using the PME method with a radial cutoff radius of 1 nm. The unit cell containing the potassium channel, membrane, and water was additionally saturated with potassium and chlorine ions at a concentration of 150 and 70 mM, respectively. This was followed by 10 ns relaxation using the above-described parameters.

The simulations of ion permeation through the potassium channel driven by a transmembrane potential were conducted using NAMD software [14]. The system simulations were carried out at six different values of electric transmembrane field strength (1500, 2250, 3000, 3750, 4500, and 6000 kcal/(mol nm e)) and a potassium salt concentration of 70 and 150 mM. The trajectory length of molecular dynamics simulations was 100 ns for each value of an external electric field strength and potassium salt concentration. The calculation parameters were chosen similar to those used in the final step of the membrane relaxation.

Molecular Dynamics Simulations of the Kv1.2 Channel Complexed with the Blocker

The simulations of the Kv1.2 channel complexed with the blocker [15] were based on the open state crystal structure of the Kv1.2 channel (PDB ID 2a79) [16] and the crystal structure of agitoxin (PDB ID 1agt) [17]. It is worth mentioning that segments S1–S4 were eliminated from the simulations for the sake of simplicity since only segments S5 and S6 play a role in the channel pore formation as well as blocker binding. Initial configurations of channel complexes with the blocker were generated manually using a Maestro molecular design software [18]. The initial position of the blocker regarding the channel was based on published data [19]. To exclude the possibility of unnecessary overlap of ions positioned within the channel with the blocker molecule, two potassium ions at sites S_0 and S_1 were removed from the channel cavity; the remaining potassium ions, including S_2, S_3, S_4, and S_{cav} retained without changes.

The obtained channel-blocker complex was further embedded in a lipid bilayer consisting of 288 molecules of palmitoylphosphatidylcholine [20]. The remained volume of the unit cell was further filled with water molecules and ions. This resulted in the formation of the unit cell with a volume of $9.62 \times 9.62 \times 10.0$ nm^3 containing one channel-blocker complex, approximately 210 lipid molecules, approximately 13500 water molecules, and the amount of sodium and chlorine ions sufficient for both compensation of the protein charge as well as for providing the desired ionic strength of the solution.

The molecular dynamics simulations were conducted in heavy atom approximation in the ffG43a2 force field using the GROMACS 4.0 program [21]. An integration time step of 2 fs was used. The simulations resulted in dynamic trajectories of the 5 ns length at a temperature of 300°C. The system characteristics were calculated using internal functions of the GROMACS program. The visualization of molecular structures and electrostatic potential calculations were carried out using the VMD program [22]. Partial atomic charges were calculated using a web-interface of the PDB2PQR program [23].

RESULTS AND DISCUSSION

Simulations of Ion Transport Dynamics through the SF of the KcsA Channel

The simulations were carried out under near physiological conditions. The root mean-square deviation (RMSD) from the initial X-ray structure over the course of molecular dynamic simulation reached a plateau at approximately 4 ns. Moreover, the maximum RMSD value was equal to 0.17 nm and is, therefore, found to be within a range of average RMSD values typical for membrane proteins (0.20 nm), suggesting that the developed model of the channel is rather stable. It should be noted that the cytoplasmic part of the channel was not present in the structure that was used for the simulations. However, the simulation results demonstrate that a partial destruction of the secondary structure of the protein C-terminus has no effect on the average pore diameter at the channel gate, which is equal to 0.50 nm. To optimize the calculation procedure, torsion angles of the selective filter (SF) backbone were restrained using a harmonic potential with a force constant of 1.5 kcal/(mol rad^2), which corresponds to a torsion angle RMSD of 51°, as recommended in [4]. The above-described model was used in further calculations.

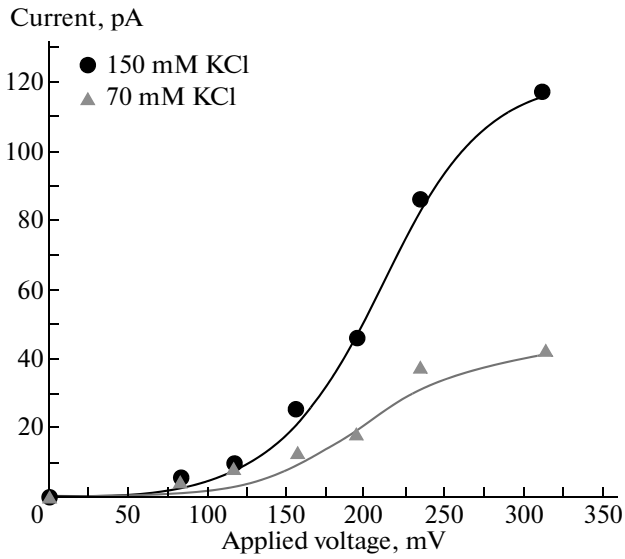

Fig. 1. Voltage-current characteristic of KcsA at a potassium salt concentration of 150 (circles) and 70 mM (triangles).

Transport of Potassium Ions across the KcsA Channel. Voltage-Current Characteristic of the Potassium Channel

The effect of thermal motion in an external electric field results in transport of potassium ions across the channel pore towards the lower values of potential. The permeation process involves quite a few stages. At first, a potassium ion proceeds into the area of the channel gate. The ion entered the channel cavity then approaches the intracellular entrance to the SF. At relatively high values of electric field strength, the selectivity filter is occupied by two potassium ions at sites S_4 and S_2, which are separated by water molecules at sites S_3 and S_1. Under the influence of an external field, the ion pushes the two potassium ions and the two water molecules in the selectivity filter towards the exit on the extracellular side. It is worthy mentioning that, at first, a molecule of water from the channel cavity enters the selectivity filter, and only then is followed by a potassium ion. In the transition state, the two potassium ions, one of which is positioned at the intracellular entrance to the SF, occupy sites S_3 and S_1, whilst the two water molecules are located at sites S_4 and S_2. The potassium ion at the entrance to the SF partially loses its hydration shell, except for two water molecules located one above and one underneath of the ion. The ion dehydration energy is compensated by the energy of ion binding with carbonyl oxygen atoms, which are positioned at the vertices of a cubic antiprism and form a solvation sphere analogue. At this stage, there are three potassium ions in the SF, which occupy the S_4, S_2, and S_0 sites, whereas the two molecules of water are located at sites S_3 and S_1. The potassium ion in S_0 restores the hydration shell and exits in the extracellular solution. Another potassium ion enters the channel cavity from the cytoplasm and the above-described process cycles again. The mechanism of ion transport obtained as a result of the performed molecular dynamics simulations is in good agreement with the mechanism described in the literature [2–4].

Voltage-current characteristics of the KcsA channel at a KCl concentration of 150 and 70 mM are represented by S-shaped curves (Fig. 1). The inflection point of the curve occurs at approximately 150 mV, where the current strength rises steeply. The dependence reaches a plateau at above 300 mV. It is noteworthy mentioning that the S-shaped dependence of the voltage-current characteristic is determined by apparent changes in the mechanism of potassium ion transport through the SF that depends strongly on the applied voltage. At high voltage values, the transport of ions across the channel SF occurs according to the above-described mechanism. In contrary, a reduction of the applied electric field strength results in dramatic changes of the ion transport mechanism. In this case, water molecules do not enter the selectivity filter, whereas the potassium ions in the SF are not separated by the binding site; moreover, more than two potassium ions can be present at the SF level simultaneously, as a result of which the current strength value drops dramatically.

Thus, a potassium ion does not necessarily follow a water molecule in the selectivity filter; it can actually permeate the SF first. The probability of this event increases with decrease of the SF voltage. It, therefore, can be expected that potassium ions that follow the above-described permeation scheme block KcsA for a certain period of time, which results in a reduction of the current strength. The hypothesis that KcsA channels inactivate as a result of the binding of potassium ions coming from the extracellular side has been earlier suggested in [24].

The maximum current strength values predicted by the molecular dynamics simulations were found to be four to five times higher than those detected experimentally [25]. Moreover, experimental voltage-current characteristic dependences reach a plateau at higher voltage values (about 400 mV) compared to those calculated in this work. It is important to mention that, in the case of saturating voltages, the strength of the current driven through the channel would be limited by both the potassium salt concentration as well as the diffusion coefficient in water. The above-mentioned differences can be explained by some disadvantages of the TIP3P water model used for the simulations in this work.

Free Energy Profiles of Potassium Ions Located in the Channel

The detection of free energy profiles of either an individual or a few potassium ions in the channel SF is of great importance for understanding the ion transport mechanism.

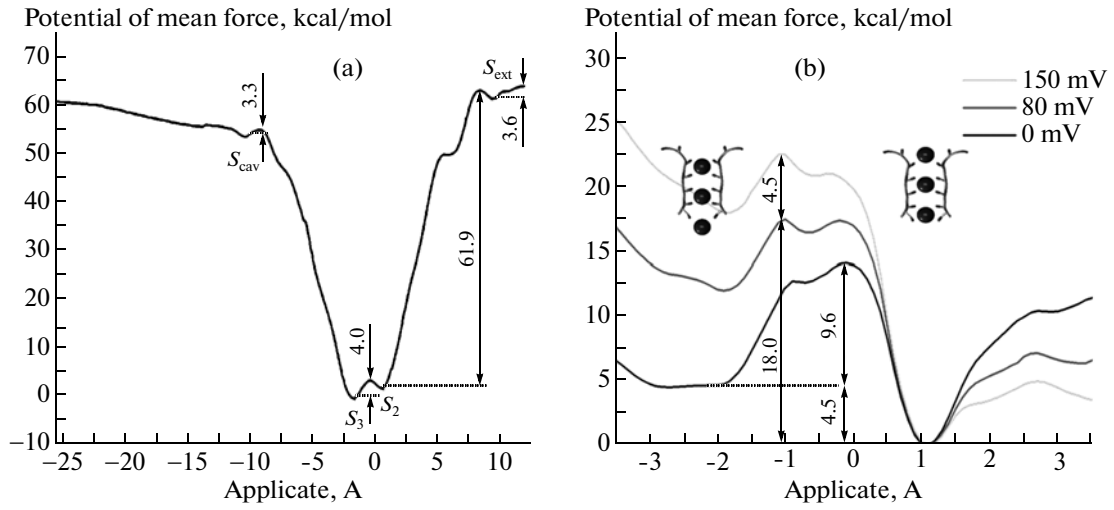

Fig. 2. Potential of mean force of (a) an individual potassium ion and (b) a three-ion configuration of the selectivity filter. (a) S_{cav}, S_3, S_2, and S_{ext} are the binding sites of an individual potassium ion in the KcsA channel. (b) Top and middle curves correspond to a system in which a voltage of 150 and 80 mV, correspondingly, was applied to the selectivity filter. Bottom curve demonstrates the situation in which no external voltage was applied. Numbers indicate energetic barriers values for permeation of an ion of potassium across the channel pore. Channel gates are located in the area of negative coordinates, whereas the selectivity filter is positioned in the region of positive coordinate values.

The observed free energy profile of an individual potassium ion in the channel clearly demonstrates the presence of four binding sites, one of which is located at the level of the channel water cavity and corresponds to the S_{cav} site, two others are positioned at the SF level and correspond to sites S_3 and S_2, whereas the last binding site is located in the extracellular solution and corresponds to site S_{ext}. The energy barriers of the ion exit from the site in the water cavity is 3.3 kcal/mol; the exit from sites S_3 or S_2 at the SF level would require an energy of 4.0 or 61.9 kcal/mol, respectively; in the case of the ion exit on the extracellular side, the energy barrier was found to be 3.6 kcal/mol. Thus, a probability that the ion would actually be able to exit the SF within the estimated time after it overcomes an energy barrier of more than 100 kT is rather low. It, therefore, can be concluded that a potassium ion, which enters the SF, stays in the channel until the external conditions change, that is, until additional ions enter the cavity from the cytoplasm or until a strong external electric field is applied to the system. It should be noted that the KcsA channel is actually occupied by potassium ions permanently, and the efficient permeation of ions through the SF is not possible in the case of individual ions. On the contrary, the mechanism is realized successfully only when two or more ions move in concert; this type of ion movement is called a single file mechanism.

The free energy profile of a potassium ion triplet in the SF is shown in Fig. 2b, where a transition between two different states of the SF can be clearly seen. In the initial state, two potassium ions occupy sites S_3 and S_1, whereas another ion is located at the entrance to the SF; following the transition, the three potassium ions populate sites S_4, S_2, and S_0. The energy barrier of the transition between these two states decreases with an increase of the voltage in the SF and is equal to 9.6, 5.9, and 4.5 kcal/mol at 0, 80, and 150 mV, correspondingly. In the absence of an external field, the 16 kT energy barrier, which is approximately six times lower compared to the largest free energy barrier of an individual ion, should be overcome by the ion triplet. At a voltage of 150 mV, the energy barrier of the ion triplet decreases to 7.5 kT. Therefore, permeation of three ions in concert through the SF (single file mechanism) seems to be much more favorable compared to the transport of individual ions.

Interaction of Voltage-Gated Eukaryotic Channels with a Blocker

In this section, we describe the effect of interionic interactions in the pore of the eukaryotic ionic Kv1.2 channel, including segments S_5 and S_6, on its binding with a blocker (neurotoxin). The pore area structure of the Kv1.2 channel is highly homologous to that of the above-described bacterial KcsA channel.

The analysis of a series of solutions of different ionic strength allowed us to conclude that the mobility of amino acid residues in the complex is ultimately a function of the secondary structure type; moreover, the changes in the solution ionic strength results in no significant effect on the motion of amino acid residues located deep inside the bilayer as well as residues in the water environment (Fig. 3a). It should be noted that a lysine residue, which plays a key role in the interactions with oxygen atoms of the main amino acid chain that form a part of the channel SF (Y445, G444), is

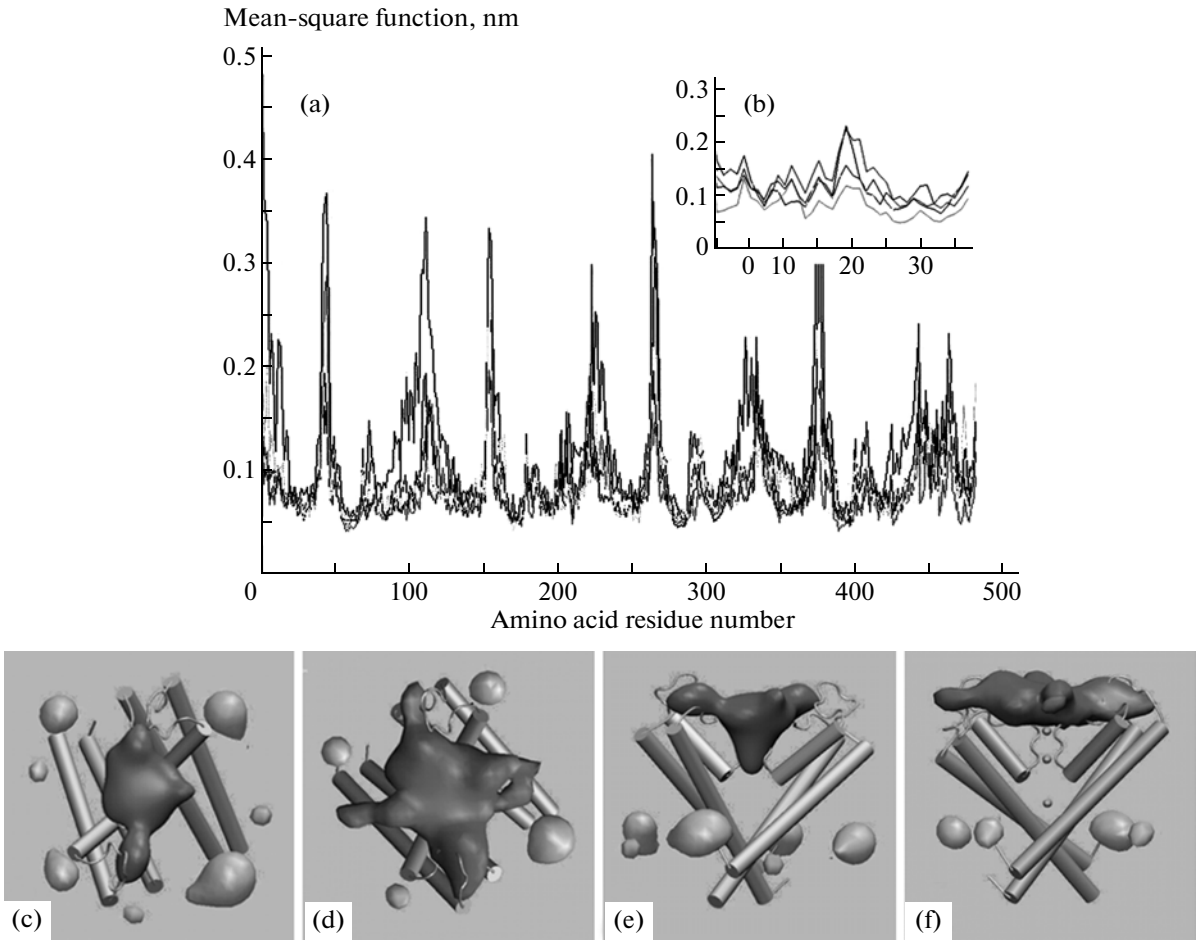

Fig. 3. Mean-square fluctuations of C_α-atoms in the protein-blocker complex. (a) Abscissa shows a number of a C_α-atom (amino acid residue number), whereas ordinate represents fluctuations of the atom. Numbers 445–482 correspond to a molecule of neurotoxin. (b) Fluctuations of atoms of the blocker. A lysine residue that plays a crucial role in binding is numbered 27. (c–f) Equipotential surfaces of the electrostatic potential of the Kv1.2 channel (c, e) with no potassium ions and (d, f) six ions in the pore and the selective filter. Ions are represented by balls. Snapshot from (c, d) the extracellular side and (e, f) the membrane plane. To improve visualization, only two out or four subunits of the channel are shown. Equiscalar surfaces are shown for the potential values equal to either 0.8MIN + 0.2MAX (negative potential, dark curve) or 0.2MIN + 0.8MAX (positive potential, light curve).

characterized by lower mobility (Fig. 3b) compared to the above-mentioned residues within the tested range of ionic strength values [26]. According to the data obtained in this work, channel fluctuations lead to the lysine side chain location outside the channel pore. This result differs from the one described in [19], where the lysine side chain remained in the pore throughout the whole molecular dynamics trajectory. The possible explanation here is that a potassium ion in the model described in [19] was excluded from the simulations, whereas the K^+ ion occupied site S2 throughout the simulations in our model, thus providing electrostatic repulsion between the potassium ion and a positively charged nitrogen atom of the K27 lysine side chain. It is worth mentioning that a very tight interaction is observed between the blocker and the channel throughout the whole simulation trajectory. Moreover, the majority of interactions shown in [19] were also observed in this work.

To estimate the effect of potassium ion presence in the SF and the central pore on binding with agitoxin, maps of the electrostatic potential, which is provided by the channel molecule in the external environment in the presence or absence of potassium ions, were prepared (Figs. 3c–3f). In the presence of potassium ions, the area of high negative potential is displaced from the SF and extends to the whole channel surface (Fig. 3e). At the same time, the area of high positive potential drifts from positively charged amino acid residues of the channel periphery to potassium ions in the central pore (Fig. 3f). Thus, filling the channel with potassium ions seems to reduce the nonselective affinity of the positively charged blocker and the binding site, which plays an obvious role at long distances. At short distances, however, when the neurotoxin and

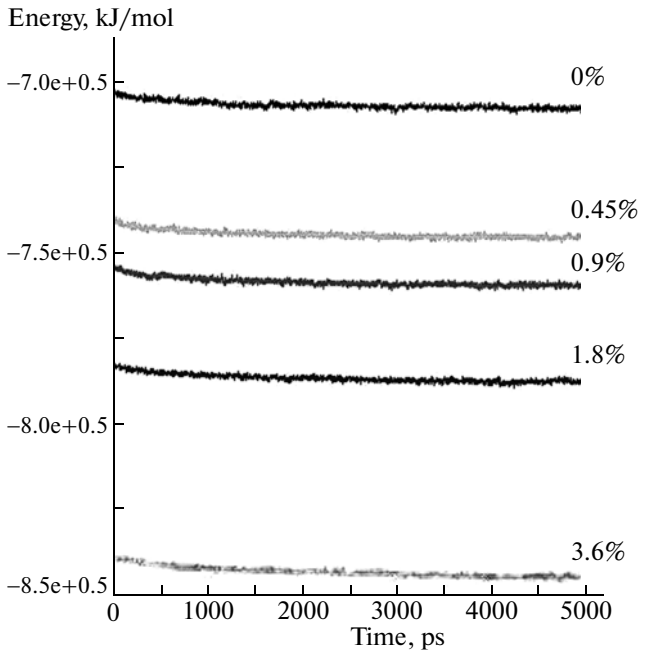

Fig. 4. Total energy profile in the investigated channel-blocker system. Influence of the ionic strength.

the binding site appear to be within a direct contact, van der Waals forces play the most important role in the process.

It is noteworthy that absolute values of total energy of the investigated systems increase simultaneously with an increase of the ionic strength (Fig. 4a), and this is simply due to an increase of the Coulomb interaction component between ions in the solution. The energy of Coulomb interactions between the protein subunits, including the interactions between the neurotoxin and the channel, change insignificantly (Fig. 4b).

CONCLUSIONS

In the present work, the full-atomic molecular dynamics model of the bacterial voltage-gated potassium KcsA channel based on the latest X-ray crystallography structure of the channel in the open state was developed. The study of the dynamics of ion permeation across the channel demonstrated the crucial role of ion–ion interactions in the channel for the ion transport process. The calculated free energy profiles for an individual potassium ion and for a few ions permeating in concert confirmed the prevalence of the single file mechanism of an ionic current, according to which two or more ions move in concert. The calculated voltage–current characteristics were shown to be in a semiquantitative agreement with experimental data.

Molecular dynamics simulations of the complex of the Kv channel with the neurotoxin showed that the evaluated model is stable, and the data about intermolecular interactions of the blocker with amino acid residues of the channel were found to be in a good agreement with the experiment. The analysis of the energy of interactions within the studied model demonstrated that the effect of the solution ion strength on binding of Kv channels with the blocker is rather insufficient. The obtained data are highly recommended to be taken into account for the studies of the affinity of channels and their mutants to various ligands.

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