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ORIGINAL PAPER

Modeling negative ion defect migration through the gramicidin A channel

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Abstract The results of potential of mean force (PMF) calculations for the distinct stages of proton conduction through the gramicidin A channel, including proton migration, reorientation of the water file and negative ion defect migration, are presented. The negative ion defect migration mechanism was hypothesized in experimental studies but was not considered previously in molecular dynamics simulations. The model system consisted of the peptide chains constructed on the base of the structure PDBID:1JNO, the inner file of nine water molecules and external clusters of water molecules placed at both ends of the channel. Potential energy functions were computed with the CHARMM/PM6/TIP3P parameters. The results obtained for proton migration and water file reorientation are basically consistent with those reported previously by *Pómès and Roux* (Biophys J 82:2304, 2002) within the similar approach. For the newly considered mechanism of negative ion defect migration from the channel center to the end of the water file we obtain the energy 3.8 kcal mol^{-1} which is not considerably different from the activation energy of water reorientation, 5.4 kcal mol^{-1}. Therefore this mechanism may principally compete for the rate-limiting step in proton conduction in gramicidin.

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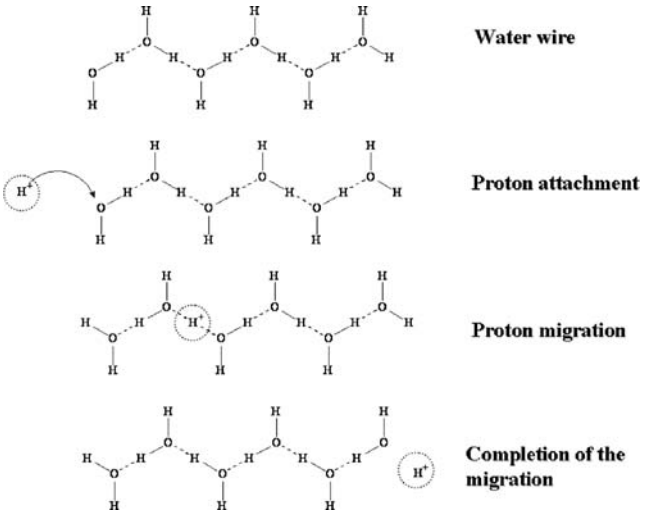
Introduction

The transmembrane ion channel gramicidin A (gA) is a head-to-head dimer each unit of which consists of 15 alternating D- and L-amino acids (L-Val L-Gly L-Ala D-Leu D-Ala D-Val L-Val D-Val L-Trp D-Leu L-Trp D-Leu L-Trp D-Leu L-Trp) capped by a formyl and ethanolamine group [1]. It is known that the proton conductance in gramicidin considerably exceeds that of any other cations. This is attributed to the feature that the inner cavity of the channel may be filled with the water molecules arranged as a hydrogen bonded single file (the water wire [2]) particularly suited for proton translocation by the Grotthuss mechanism [3]. In spite of multiple studies on the gramicidin conductance there is no common agreement on what is the rate-limiting stage of the process.

To explain the observations that the increased membrane dipole potential and decreased peptide-chain dipoles facilitate proton transport Philips et al. [4] assume that: (1) proton transport through the gA channel occurs by means of the Grotthuss mechanism, (2) water reorientation after proton translocation is the rate-limiting stage of the process, (3) reorientation of the water file is initiated at the channel exit. In more recent studies the effects of agents modulating the membrane dipole potential Rokitskaya et al. [5] found that the proton and potassium conductance exhibited changes in opposite directions in response to changes in the membrane dipole potential. To interpret these observations the authors put forward an interesting model that the negative charge movement may be one of the rate-limiting stages for



Scheme 1 Illustration of the proton migration mechanism

proton translocation in gA. However no support of this hypothesis was provided in subsequent studies.

From the theoretical side, the molecular dynamics (MD) based simulations of proton transport in ion channels [6–13] play an important role in determining the details of its mechanism. In particular, the authors of studies [6–8] emphasized important aspects of the "hop-and-turn" mechanism in gA at the molecular level. The "hop" steps correspond to the translocation of the cationic defect, H^+, from end to end of the water wire *via* the Grotthuss mechanism. The reorientation of the water file constitutes the "turn" stage which may be also considered as the directional migration of a bonding defect in the hydrogen bond network. According to the calculations of free energy profiles carried out by *Pómès and Roux* for the polar model of the gA channel [8] the reorientation of the unprotonated water file proceeds with the activation energy approximately four times larger than the energy required to move a proton from the minimal energy point at the center of the water file to the end. In the paper by Braun-Sand et al. [12] an assumption that the orientation of the unprotonated water file is rate limiting is called problematic. By the results of modeling with the empirical valence bond (EVB) potential energy functions the authors advocate that the proton transfer process in gA is controlled by the barrier associated with the electrostatic energy of the transferred proton rather than by the water orientational effects [12].

In this work we compare free energy profiles computed for the distinct stages of proton conduction through the model gA channel following a direction outlined primarily in the works by *Pómès and Roux* [6, 8, 13]. The main objective of the study is to estimate performance of the negative ion defect migration mechanism [5] which has not been considered in previous theoretical papers.

Methods

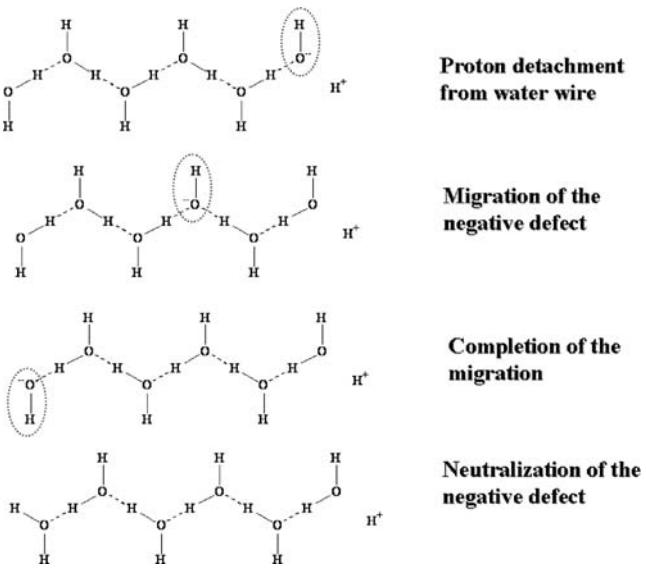
The proton migration and the negative defect migration mechanisms are illustrated in Schemes 1, and 2.

According to the hypothesis [5] the migration of OH^- may be associated with one of the rate limiting stages of proton conduction through gramicidin.

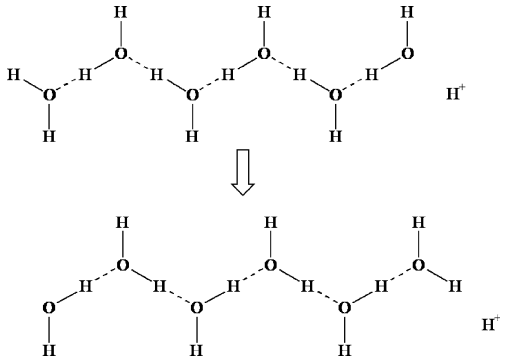
The reorientation of the water file inside the channel which is necessary to complete each cycle of conduction and to prepare the system to the next episode is illustrated in Scheme 3.

To characterize these stages from the energetic perspective we performed calculations of free energy profiles, or the potential of mean force (PMF), for the transformations illustrated in Schemes 1, 2 and 3 by using the molecular modeling methods.

The model system considered in this work is shown in Fig. 1. We started from the atomic coordinates corresponding to the gA structure 1JNO [14] from the Protein Data Bank archive. After the hydrogen atoms were added and the file of nine water molecules was introduced in the cavity the geometry parameters of the model system were optimized in the flexible effective fragment potential quantum mechanical – molecular mechanical (QM/MM) calculations as described, e.g., in [15]. This structure was solvated by placing it at the center of a cubic box 40×40×40 $Å^3$ of water and equilibrated at temperature 500 K by the 1 ns molecular dynamics simulations with the AMBER force field parameters [16]. After cooling down to 300 K the system was again equilibrated for 1 ns. For subsequent free energy calculations, beyond nine water molecules that occupied the inner cavity of gA, two clusters of 20 water



Scheme 2 Illustration of the negative ionic defect migration mechanism



Scheme 3 Illustration of the water file reorientation stage

molecules at both ends of the channel were kept in the model system as illustrated in Fig. 1.

In calculations we used an original computer code that realized the constant temperature molecular dynamics simulations for the canonical (NVT) ensemble in conjunction with the Nose-Poincare thermostat [17, 18]. The umbrella sampling technique [19] and the weighted histogram analysis method [20] were applied for PMF estimates. The potential energy functions were constructed by combining the polarized model (PM6) empirical parameters for water molecules in the wire [21], the all-atom CHARMM force field parameters [22] for the peptide walls of the gA channel and the TIP3P potential [23] for the outer water molecules. Application of the PM6 potential, which assumes the H, O, H^+ and O^{2-} particles representing the H_2O molecule, allows one to account for cleavage and formation of the O-H bonds in water molecules as well as hydrogen bonds in water clusters and to model transformations illustrated in Schemes 1, 2 and 3.

The potential of mean force (PMF) should be calculated for the equilibrated system as a function of the reaction coordinate which is defined as in Ref. [8] as the projection of the dipole moment onto the channel axis, $\mu_z=\sum q_i z_i$, with the charges $q_O=-2.0\ e$, $q_H=1.0\ e$. As recommended in [8] we considered

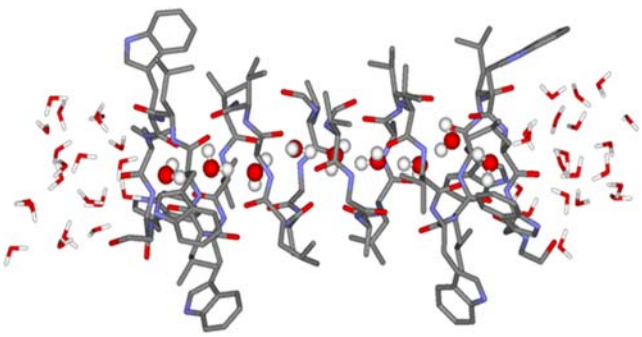


Fig. 1 Model structure of the gA channel with the inner file of nine water molecules distinguished in the balls-and-sticks representation. Hydrogen atoms of the peptide walls are not shown

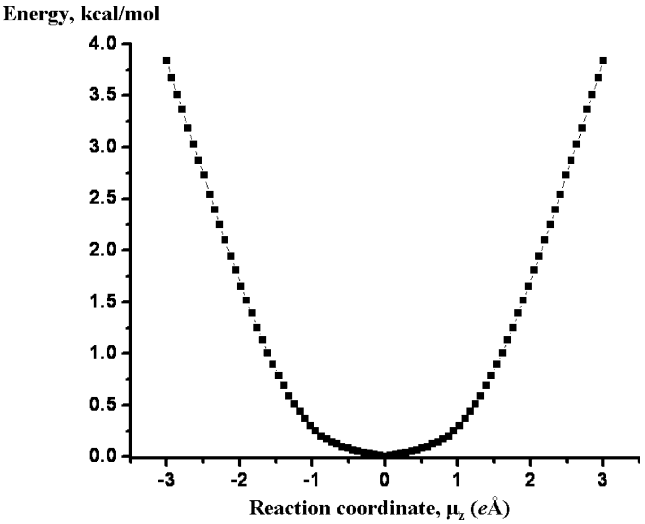


Fig. 2 The computed free energy profile for migration of the negative ion defect according to Scheme 2

explicitly the range of μ_z from 0 to 3 e•Å for modeling migration of the charged species (Schemes 1, and 2) from the center of channel to its end and the range from 0 to 8 e•Å for the half-reorientation of the water file. The symmetry related parts of the graphs corresponding to the ranges (0; −3 e•Å) and (0; −8 e•Å) were constructed as mirror images, what allowed us to cover the entire migration region inside the channel and the complete re-arrangement of the water file inside the channel.

The explicitly considered ranges of the reaction coordinate were divided into 6 windows (5 windows for the reorientation of the water file) with the harmonic restraining potential with

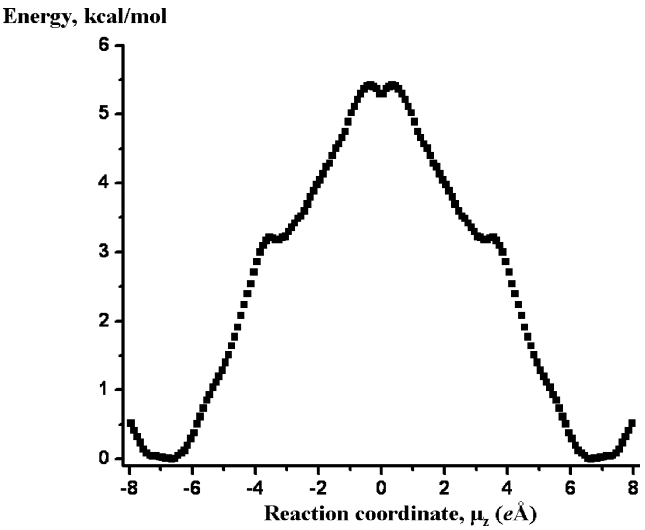


Fig. 3 The computed free energy profile for the reorientation of the chain of nine water molecules (balls and sticks in Fig. 1) inside the channel

the force constant 2.0 kcal mol^{-1}•(e•Å)$^{-2}$. In each window, the configurations were sampled for 100 ps after 20 ps for equilibration at 300 K with the integration time step 1 fs.

Results

To model the proton migration stage (Scheme 1) we added a proton to the file of nine water molecules at one end (balls and sticks in Fig. 1) and computed the PMF for its translocation to another end of the file. The computed graph presents a smooth parabolic-like curve with a minimum corresponding to the residence of extra proton on the central water molecule in the file of nine species. The amount of free energy required to transfer extra proton from the position of minimum to the end of the water file was estimated to be 2 kcal mol^{-1}.

To model migration of the negative ion defect (Scheme 2) we detached a proton from the water chain at one end (balls and sticks in Fig. 1) and computed the PMF for migration of the OH^- species to another end of the file. The computed graph (Fig. 2) also presents a smooth parabolic-like curve with a minimum corresponding to the residence of OH^- at the center of the inner chain of water molecules. The amount of free energy required to transfer the defect from the center to the end of the water file was estimated to be 3.8 kcal mol^{-1}.

The computed graph of the PMF for the reorientation of the inner chain of nine water molecules (balls and sticks in Fig. 1) is presented in Fig. 3. According to these calculations this stage is characterized by the highest activation free energy barrier of 5.4 kcal mol^{-1}.

Conclusions

The stages of proton migration and of water file reorientation by using the CHARMM program [24] and the PM6 water potentials were considered previously by *Pómès and Roux* [8, 13]. Their computed PMF for slightly different molecular models of the channel and for a slightly different computational protocol amounted to the activation barriers of 1.1 kcal mol^{-1} for the proton migration (~4 kcal mol^{-1} for the process in the nonpolar analog of gA) and of 3.8 kcal mol^{-1} for the water reorientation (7.6 kcal mol^{-1} in the nonpolar channel). The process of negative ion defect migration was not considered in previous works.

The results obtained here for proton migration and water file reorientation are basically consistent with those reported previously by *Pómès and Roux* for the polar channel [8]. The experimentally determined activation free energy of the proton conductance in gramicidin channels from the observed current/voltage relationship is reported to be 6.5 kcal mol^{-1} [25], which is close to the value computed here for the stage of the reorientation of the water file inside the channel (5.4 kcal mol^{-1}).

For the newly considered stage of negative ion defect migration we report the energy amount required to propagate the defect from the channel center to the end of the water file (3.8 kcal mol^{-1}) which is not considerably different from the activation energy of water reorientation (5.4 kcal mol^{-1}). Therefore the negative ion defect mechanism may principally compete for the rate-limiting step as hypothesized by Rokitskaya et al. [5] especially in the modified gramicidin channels.

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