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Diffusion constant of K^+ inside Gramicidin A: A comparative study of four computational methods

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Abstract

The local diffusion constant of K^+ inside the Gramicidin A (GA) channel has been calculated using four computational methods based on molecular dynamics (MD) simulations, specifically: Mean Square Displacement (MSD), Velocity Autocorrelation Function (VACF), Second Fluctuation Dissipation Theorem (SFDT) and analysis of the Generalized Langevin Equation for a Harmonic Oscillator (GLE-HO). All methods were first tested and compared for K^+ in bulk water—all predicted the correct diffusion constant. Inside GA, MSD and VACF methods were found to be unreliable because they are biased by the systematic force exerted by the membrane-channel system on the ion. SFDT and GLE-HO techniques properly unbias the influence of the systematic force on the diffusion properties and predicted a similar diffusion constant of K^+ inside GA, namely, ca. 10 times smaller than in the bulk. It was found that both SFDT and GLE-HO methods require extensive MD sampling on the order of tens of nanoseconds to predict a reliable diffusion constant of K^+ inside GA.

Keywords: Ionic diffusion coefficient; Molecular dynamics simulation; Gramicidin A; Potassium

1. Introduction

There is a great deal of interest in studying biological ion channels due to the important roles that they play in the physiology of organelles, cells and tissues. With the availability of detailed atomistic structures of several ion channels (Gramicidin A (GA) [1], KcsA potassium channel [2], α-hemolysin [3], ClC chloride channel [4]) it has become feasible to do accurate theoretical modeling of ion currents in order to understand the mechanisms of ion transport through biological channels. At present, the most popular methods of ion current modeling are Poisson–Nernst–Planck (PNP) [5–10], Brownian Dynamics (BD) [11–16] and Non-equilibrium Molecular dynamics (NEMD) [17–19]. Of these methods PNP is the most primitive but fastest method. In PNP, ions are represented by continuous densities whose steady state concentrations are calculated in the electrostatic field due to partial charges on the protein and mobile ion charge densities, plus a contribution due to external electrodes, by solving Poisson's equation self-consistently with a Nernst–Planck equation for each ion species [5]. In BD, ions are modeled explicitly but water is treated implicitly as a continuous medium characterized by dielectric and friction constants. In BD, ions move in the electrostatic field of partial charges on the protein, surface charges induced on dielectric boundaries within the system, externally applied electric fields, pairwise electrostatic interactions with other ions and steric overlap interactions with other ions and the walls of the protein/membrane system [12]. In NEMD the entire system, including water, is modeled explicitly and the dynamics of all atoms is computed by numerical integration of Newton's second law using an atomistic force field [17–19]. Therefore, NEMD is the most accurate method, but very slow compared to PNP and BD and still not very practical.

For calculating ion currents, both PNP and BD methods rely heavily on the magnitude of the diffusion constant inside the channel, which is a phenomenological input into these theories. To date, there are no direct experimental measurements of diffusion constants of ions inside narrow pores. Therefore, one must rely on simulations to predict diffusion constants, and,

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indeed, several theoretical methods have been developed for this purpose. They are widely used for calculating diffusion properties of ions and molecules in bulk phases [20–22], but the applicability of some of these methods to narrow ion channels (e.g., Gramicidin A) is questionable. Currently, there is no consensus in the biophysics literature about the magnitude of diffusion constants of ions inside narrow channels [6,23–28]. Different methods and authors have predicted a wide range of diffusion constants. Therefore, it is imperative to test and compare different methods to assess their applicability in narrow channels and to estimate the value of the diffusion constants of ions inside such channels.

An important question that has to be addressed first is how to define the diffusion constant. In fact, the diffusion constant can be defined in many different ways depending on the model used to describe transport of ions across the channel. In Brownian (Smoluchowski) Dynamics and PNP-like models the flux $\vec{j_i}(\vec{r},t)$ of ion species i is expressed as

$$\vec{j_i}(\vec{r},t) = -D_i\left[\vec{\nabla}c_i(\vec{r},t) + c_i(\vec{r},t)\vec{\nabla}(\beta\psi_i(\vec{r}))\right], \qquad (1)$$

where D_i is the diffusion constant for this species, $c_i(\vec{r},t)$ is its concentration and $\psi_i(\vec{r})$ is its free energy or potential of mean force (PMF); furthermore, $\beta = \frac{1}{k_B T}$ (k_B is Boltzmann's constant and T is absolute temperature). The free energy of an ion at a given position in space arises from its interactions with the protein, membrane, and water molecules. In particular, these interaction forces can be attributed to electrostatic interactions of the ion with the partial charges of the protein and membrane, rotational polarization of water, rotational/translational polarization of protein and membrane groups as well as electronic polarization of the protein, membrane and water. It has been shown in several studies that translational/rotational polarization of protein groups is important in electrostatic stabilization of ions inside narrow channels [6,29]. This is manifested in the flexibility of key protein groups that relax locally around the ion and stabilize it, ultimately rendering permeation more favorable.

Let us briefly review what has been done to date to calculate diffusion constants of ions inside narrow channels. The most widely employed methods for calculating diffusion constants are based on extracting the mean square displacement (MSD) or the velocity autocorrelation function (VACF) from MD simulations. In Ref. [23], the diffusion constants inside smooth cylindrical channels with repulsive walls of different width and length were calculated using the MSD method for Na^+, K^+, Cs^+, Ca^{2+}, F^-, Cl^- and I^- ions. It was observed that the diffusion constants decreased as the radius of the channel decreased. In a 3 Å radius channel the diffusion constant of K^+ was found to be ca. 5 times smaller than in the bulk water. This decrease was attributed to two main factors, one being an increase in the mean square of random forces on the ions as the channel gets narrower and the second an increase in time scale of random force correlations. In Ref. [24], the diffusion constants of K^+ and Na^+ were estimated using the MSD method from MD simulations in hydrophobic cylindrical channels with varying radii, as well as in the KcsA potassium channel. In a 3 Å radius hydrophobic channel the diffusion constants for both K^+ and Na^+ were ca. 12% of the bulk value. In Ref. [25] mobilities of K^+ and Cl^- were studied by extracting MSD and VACF functions from MD simulations inside five different channels with radii ranging from 2 Å to 6 Å. It was found that the diffusion constants were 2–10 times smaller than in the bulk solution depending on the channel width and the position where the probe ion was released. In a 2 Å radius channel the diffusion constant was found to be on average 10 times smaller than in the bulk. In Ref. [30], friction coefficients of K^+ and Na^+ ions were evaluated by fitting the analytical expression for the VACF of a Brownian harmonic oscillator to the VACF obtained from MD simulations inside the KcsA potassium channel. The authors of this study found diffusion constants of K^+ and Na^+ ca. 3 times smaller inside the channel. In Ref. [28] the effective diffusion constant of K^+ and Na^+ ions was estimated inside a Gramicidin-like β-helix using two methods. The first method utilized the effect that the dependence of the terminal velocity on the external weak force applied to the ion is proportional to the diffusion constant. The other methods used in Ref. [28] were based on the second fluctuation dissipation theorem. Both methods predicted that the effective diffusion constant of K^+ is 3–5 times smaller inside the β-helix compared to the bulk value. In our earlier study [6] the diffusion constant of K^+ inside the Gramicidin A (GA) channel was calculated using the fluctuation dissipation theorem by extracting the force autocorrelation function (FACF) from MD simulations. A reduction of 8.5 times in diffusion constant compared to the bulk value was found inside the channel.

A different approach to calculation of diffusion constants is based on fitting the diffusion constant to reproduce experimental ion currents using BD or PNP. In Ref. [27] the potential energy well depth and barrier height as well as the internal diffusion constant were fit for the GA channel: a best fit was obtained when the diffusion constant inside the channel was taken to be 10 times smaller than in the bulk. It was found that the model did not reproduce the experimentally observed saturation of ion current with ionic concentration when the diffusion contant of K^+ inside the channel was larger than 0.3 times the bulk value, implying that the value of this constant may critically influence the saturation properties of the channel. In Ref. [7] the internal diffusion constant of Cs^+ (K^+) had to be decreased 11 (17) times compared to the bulk in calculations of ion currents via the PNP model in order to get agreement with experimental results. The overall conclusion drawn from these studies is that diffusion constants of ions in narrow channels are roughly 3–10 times smaller than in the bulk. In contrast to this conclusion, it was found in Ref. [26] that the diffusion constant of K^+ inside the GA channel is not much different from the bulk. These authors used MD simulations of K^+ restrained with a harmonic potential and mapped this microscopic dynamics to the generalized Langevin equation (GLE). They estimated that the internal diffusion constant was 66% of its bulk value.

The goal of the present study is to calculate the diffusion constant of K^+ ion inside the GA channel using four different methods based on MD simulations. The paper is organized in