Size effects on the open probability of two-state ion channel system in cell membranes using microcanonical formalism based on gamma function

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Abstract. Ion channel systems are a class of proteins that reside in the membranes of all biological cells and forms conduction pores that regulate the transport of ions into and out of cells. They can be investigated theoretically in the microcanonical formalism since the number of accessible states can be easily evaluated by using the Stirling approximation to deal with factorials. In this work, we have used gamma function (\(\Gamma(n)\)) to solve the two-state or open-close channel model without any approximation. New values are calculated for the open probability \(p_0\) and the relative error between our numerical results and the approximate one using Stirling formula is presented. This error \(\left(\frac{p_{app0} - p_0}{p_0}\right)\) is significant for small channel systems.

1. Introduction
The interest in ion channel systems of all biological cells has increased greatly. They are a class of proteins that reside in the membranes of the cells and forms conduction pores that regulate the transport of ions into and out of cells [1]. One of their important functions is the conduction of action potentials in neurons. The equilibrium value function or the probability of finding a channel in the open state at equilibrium is crucial in the channel kinetics for fitting the experimental data. The probability is generally described by the Boltzmann statistics [2] and shows a direct proportion with the membrane potential. Boltzmann curves are sigmoidal-shaped curves on linear plot of open probability against potential. They are proved to be adequate for describing the steady-state data from many experiments [3]. So far, a more detailed theoretical explanation of the curves based on the free energy minimization, generalized statistical mechanics, and Monte-Carlo simulation technique has already been published by us [4, 5, 6]. In the previous work [4], we have defined the entropy in the microcanonical ensemble in terms of open and closed channel populations and used the Stirling approximation to deal with the factorials. Then we have minimized the free energy to derive the voltage-dependent open state probability of a single channel.

In the present work, we propose a new methodology to define the equilibrium value function for fitting the experimental data found during the measurements on small number of specific channel clusters in excitable membranes [7]. The methodology is again based on the
microcanonical formulation in statistical physics approach but it simply uses the definition of gamma function instead of Stirling approximation.

2. The Model and Method
We consider a two-state (or open-close) channel system in which each channel is opened by the movement of a single gating particle which carries a charge. At any moment, the particle is in one of two positions, 1 and 2, and these are associated with closed and open states, respectively. The positions 1 and 2 correspond to two wells (with energy levels $\varepsilon_1$ and $\varepsilon_2$) in energy profile, and there is a single energy barrier between them. A simple expression for the internal energy of such a system in the presence of a membrane potential ($V$) is given by

$$E = n(1 - p_0)\varepsilon_1 + n p_0 \varepsilon_2 + n q e_0 (1 - p_0) V$$ \hspace{1cm} (1)

where $n$ is total number of channels, $e_0$ elementary electronic charge and $q$ is the number of charges on gating particle. In Eq. (1), $p_0$ and $1-p_0$ are the open and closing probabilities of single channel, respectively. The number of accessible states of the system is defined by

$$\Omega = \frac{n!}{[n(1-p_0)]!(np_0)!}.$$ \hspace{1cm} (2)

Based on the Stirling approximation ($\ln n! \cong n \ln n - n$), we obtain the familiar Boltzmann entropy ($S = k \ln \Omega$) as

$$S = -nk \left[(1 - p_0) \ln (1 - p_0) + p_0 \ln p_0\right]$$ \hspace{1cm} (3)

where $k$ is the Boltzmann constant. In the following, we call Eq. (3) as the approximated entropy. The approximated Helmholtz free energy is given by the thermodynamic relation $F = E - TS$, where $T$ is the temperature. Using the minimization condition ($\partial F/\partial p_0 = 0$) one obtains the channels open probability for the static properties as follows:

$$p_0^{\text{app}} = \left\{1 + \exp \left[-\frac{q e_0}{kT} (V - V_0)\right]\right\}^{-1}$$ \hspace{1cm} (4)

where the superscript $\text{app}$ denotes approximation and $V_0$ [equal to $-(\varepsilon_1 - \varepsilon_2)/q e_0$] is the voltage at which half of the channels are open. Eq. (4) gives a sigmoidal shaped curves which start from zero and increase gradually to one as the membrane potential rises. In the literature, most of the data available from experiments done at different temperatures and in different axons were fitted using Eq. (4). From the experimental recordings made at several bath temperatures, Boltzmann parameters (such as $q$ and $V_0$) can easily be determined [2].

On the other hand, the use of Stirling approximation is generally justified in the thermodynamic limit ($n \to \infty$). But, it generates significant errors with respect to the solution using gamma function for small systems [8]. From the definitions of gamma function (GF) ($\Gamma(n+1) = n!$), we can write a value for the number of accessible states as

$$\Omega_{\text{GF}} = \frac{\Gamma(n+1)}{\Gamma[n(1-p_0)+1] \Gamma(np_0+1)}.$$ \hspace{1cm} (5)

Then, entropy ($S_{\text{GF}} = k \ln \Omega_{\text{GF}}$) becomes

$$S_{\text{GF}} = k \left\{\ln \Gamma(n+1) - \ln \Gamma[n(1-p_0)+1] - \ln \Gamma(np_0+1)\right\}.$$ \hspace{1cm} (6)

The free energy is now calculated by the formula

$$F_{\text{GF}} = E - TS_{\text{GF}}.$$ \hspace{1cm} (7)
Using the condition $\partial F/\partial p_0 = 0$ we can finally obtain the following steady-state equation for the channel opening:

$$
\psi^0 (np_0 + 1) = \psi^0 (n - np_0 + 1) + \frac{qe_0}{kT} (V - V_0)
$$

where $\psi^n (x) = \frac{d^{n+1}}{dx^{n+1}} \ln \Gamma (x)$ with $\psi^0 (x) = \frac{d}{dx} \ln \Gamma (x)$ as in Refs. [8]. It is now possible to establish a relationship between the value of open probability ($p_0$), and the number of channels in the membrane. From the solutions of Eq. (8) we have plotted $p_0$ vs. $V$ for several number of channels in Fig. 1. Also, we have calculated the relative error $(p_0^{app} - p_0)/p_0$ as functions of the membrane voltage and channel number in Figs. 2 and 3, respectively. We will briefly discuss the figures in the next section.

3. Results and Discussion

In our calculations, we have considered the voltage-gated potassium channels from xenopus oocytes [9]. Using several values of $n$, the value of probability of channel opening as function of the applied voltage is shown in Fig. 1 for the experiment illustrated in Ref. [9] with the parameter values $V_0 = -42$ mV, $kT/qe_0 = 4.8$. From the figure one can see that equilibrium value function gives a sigmoidal-shaped curve on linear plot of $p_0$ against $V$, i.e. it rises to 1 from zero as the value of $V$ grows, as in the previous works [3, 4, 5, 6]. This figure also shows the effect of number of channels on $p_0$. An increase in the number of channels located on the membrane alters the steepness of the $p_0 - V$ relation at midpoint potential ($V_0$) being smaller with higher values of $n$ and the solution of Eq. (8) reduces to one using Stirling approximation or Eq. (4) when $n \to \infty$ as expected. This corresponds to a sharp increase of the probability for opening in an ensemble of many potassium channels (or high channel densities) observed at potentials around $V \approx -60$ mV. In contrast, at low channel densities the chances for finding channels in open state shift to more negative potentials below $V = -60$ mV. The relative error $(p_0^{app} - p_0)/p_0$ is depicted in Fig. 2 as a function of $V$ for four different sizes ($n = 5$, $n = 10$, $n = 10^2$, $n = 10^3$). For membranes with very small number of channels $n < 100$, the difference between the approximated and new open probability values is relevant in the voltages below $V = -40$ mV. For larger membranes with $n > 10^3$ channels the error is lower but it is still relevant for very low voltages. In Fig. 3, the relative error is also shown in terms of system size ($n$) for different values of voltages ($V = -50, -55, -60, -70, -75, -85, -90$ mV). It becomes clear from the figure that for sizes...
Figure 2: Open probability relative error of the two-state ion channel system in terms of the membrane potential. The different curves are for four sizes.

Figure 3: Open probability relative error of the two-state ion channel system in terms of the system size. The different curves are for seven membrane voltages.

smaller than \( n = 10^2 \) the difference between the approximated and new open probabilities can be significant.

4. Conclusion
In this work, a two state (open-close) ion channel model of biological membranes has been solved. The same system has already been considered in microcanonical ensemble using Stirling approximation in many references. We have presented an analysis of the model in the microcanonical formalism without any approximation but using gamma function and its derivatives. From this analysis we conclude that we observe an excellent matching of our calculations and the one using Stirling approximation in the limit \( n \to \infty \). Above result is also consistent with the experimentally observed data at high channel densities. According to the voltage-clamp data [9], the mechanism of membrane potential stabilization is determined at high channel densities by the activation-deactivation rates while it is only affected by the inactivation process at low densities with small number of channel populations. It other words, the characteristics of voltage fluctuations for small number of channels reflect the competition between voltage-dependent rates of transition and the voltage-independent one [10]. This leads to a noticeable difference between two approaches at lower potential regime for smaller sizes (\( n < 100 \)). From the conceptual viewpoint, this is known as a lower bound to the Stirling approximation just as in Ref. [8]. Our results also develop a useful approach in the steady-state studies of channels that are not voltage-gated.
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