Effects of hydrophobic mismatch and spontaneous curvature on ion channel gating with a hinge

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We analyze the energetics of an ion-channel gating, focusing on effects of hydrophobic mismatch between the channel protein and the nearby lipid molecules, spontaneous curvature of monolayers, and thickness change of membranes. For the analysis we consider recently proposed open and closed conformations of a potassium channel which has a gating hinge, using the elastic continuum model of membranes. Gating energy, defined as the difference of deformation free energies for open and closed conformations, is quantitatively evaluated for various values of moduli related to the deformation of membranes and spontaneous curvature of monolayer imposing a strong hydrophobic boundary condition. We show that the gating mechanism with a hinge can work successfully even in a continuum model that considers hydrophobic mismatch and spontaneous curvature. When the energy cost for the thickness change of the membrane is neglected, the surface tension is not necessarily strong enough to open the channel. Otherwise, a relatively strong surface tension is required to open the channel.

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Ion channels are composed of membrane proteins. These channel proteins change their conformations during gating processes responding to various kinds of stimuli which are typically chemical, electrical, or mechanical. In an open state, ions flow through the channel with a quite rapid rate. On the other hand, the flow of ions is blocked in a closed state. The conformational changes of the channel proteins in gating processes have played an important role in understanding ion channels. Recently, open-pore conformation of the potassium channel has been proposed by electrostatic calculations [1], yielding that there is a gating hinge deep within the membrane just below the selectivity filter and the part of the channel below the gating hinge bends about 30°. Hence in the bent conformation the channel stays open widely and in the straight conformation it is closed as shown in Fig. 1. A similar gating mechanism mediated by membrane tension, so-called, gating-by-tilt of mechanically sensitive membrane channels has also been proposed recently assuming that the channel proteins are tightly attached to the edge of the membrane [2] instead of the middle. In their work the deformation free energy of the membrane has been obtained considering only curvature rigidity and surface tension of the membrane in the continuum model, yielding that the gating-by-tilt mechanism as well as the dilational gating model [3] is successful for a sufficiently large membrane tension.

The importance of membrane deformation to the gating process has been emphasized in recent experimental works of Lee and MacKinnon [4] and Suchyna *et al.* [5] in which they investigated independently how toxins interact with ion channels to block their functions. Remarkably they found that toxins do not block the channels directly but do indirectly by partitioning into the membrane (so deforming the membrane).

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Motivated by the above works [1,2,4,5], we analyze the energetics of the gating mechanism illustrated in Fig. 1 by considering the membrane as an elastic continuum [6]. The continuum model is based on the property of membrane elasticity and is well known to be effective in understanding gating processes occurring in a quite slow time scale of milliseconds. In this work we focus on the effect of a strong hydrophobic interaction between the channel proteins and the nearby lipid molecules. The hydrophobic exterior length of channel proteins usually does not match the hydrophobic thickness of the membrane. Hence the hydrophobic part of proteins or membrane lipids can be exposed in water during gating processes. This hydrophobic mismatch makes the membrane and proteins frustrated and deformed so that it cannot be neglected. Hence we impose a strong hydrophobic boundary condition at the interface between the ion channel and membrane. We also investigate the role of the spontaneous curvature of the monolayer to the gating. The lipid concentration in the outer layer of the membrane is not necessarily equal to that in the inner layer. Such a difference in lipid concentration can be introduced in the continuum model by imposing a spontaneous curvature of the monolayer. The importance of the lipid concentration on the gating of MscL channels has been emphasized in a recent experiment [7]. Both effects of the hydrophobic mismatch and the spontaneous curvature of monolayer are discussed not only in the absence but also in the presence of the thickness change of the membrane.

The hydrophobic mismatch between channel proteins and membrane bilayers during the gating process perturbs the packing of lipid molecules in the vicinity of the channel. The deformation free energy for this perturbation can be expressed by three ingredients related to changes of the thickness, curvature, and surface area of the membrane [8]. These changes of the membrane are described by the elastic deformation modulus κ_{th}, mean splay-distortion modulus κ_{cv}, and surface tension κ_{st}, respectively. For a sufficiently small deviation $u(\vec{r})$ normal to an unperturbed plane, we describe the effective deformation free energy of each layer as a surface

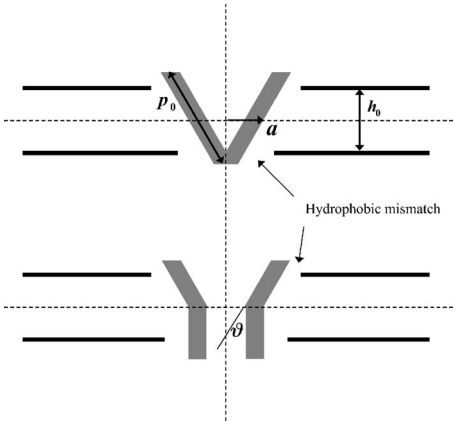

FIG. 1. Schematic images of closed and open states for the potassium channel. The gray parts represent the channel proteins. A gating hinge is located approximately in the middle of the membrane. The channel is closed in the straight or conical conformation and open in the bent or bottleneck conformation. Disconnected regions around the channel are frustrated by a hydrophobic interaction. The upper (lower) line in each image denotes the outer (inner) layer of the membrane.

integral over the perturbed area to second order:

$$F = \frac{1}{2}\int_{r_0}^{\infty} 2\pi r dr \left\{ \frac{\kappa_{th}}{h_0^2}u^2 + \kappa_{cv}(\nabla^2 u - C_0)^2 + \kappa_{st}(\nabla u)^2 \right\} - \frac{1}{2}\int_{r_0}^{\infty} 2\pi r dr(\kappa_{cv}C_0^2), \quad (1)$$

where $r(=|\vec{r}|)$ is the radial distance, ∇ is the plane polar gradient, r_0 is the position of the interface between the channel and membrane, h_0 is the thickness of the unperturbed membrane, and C_0 is the spontaneous curvature of the monolayer. The last term is the curvature frustration energy originating from the shape changes of lipid molecules to form the unperturbed bilayer [9,10]. Since we are interested in the deformation energy with respect to the unperturbed membrane, the curvature frustration energy exactly cancels the C_0^2 term in the first integral and so the whole integral is convergent. Note that Eq. (1) is valid only for a weak perturbation of∇u and $u(r)$ is not symmetric about the midline of the membrane due to the lack of symmetry of our systems. In this work we do not discuss the contributions of the channel deformation and area change to the total free energy.

For a given set of moduli, a stationary deformation $u_s(r)$ minimizes the free energy cost of deformation. Hence minimizing the deformation free energy in Eq. (1) with respect to variations in u, we obtain the Euler-Lagrange equation

$$\nabla^4 u_s - \frac{1}{\beta}\nabla^2 u_s + \frac{\alpha}{\beta h_0^2}u_s = 0, \quad (2)$$

where $\alpha=\kappa_{th}/\kappa_{st}$ and $\beta=\kappa_{cv}/\kappa_{st}$. Note that α is dimensionless and β has the dimension of area, Å^2. It is important to notice that the above equation does not depend on the spontaneous curvature C_0. Hence the stationary solution u_s is invariant under C_0 though the deformation free energy is variant. It is well known that the stationary solution can be expressed as a linear combination of the modified Bessel functions of the second kind, K_0 [11], if imposing boundary conditions at $r\to\infty$—such as, $\lim_{r\to\infty} u_s(r)=0$ and $\lim_{r\to\infty}(du_s/dr)=0$,

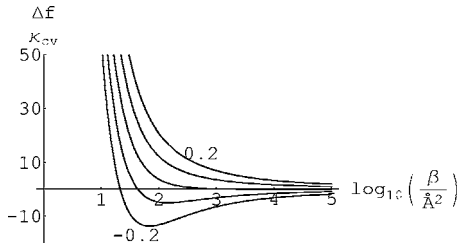

FIG. 2. The gating energy $\Delta f/\kappa_{cv}$ as a function of $\log_{10}(\beta/\text{Å}^2)$ when $\alpha=0$. The graphs refer to five values of the spontaneous curvature corresponding to −0.2, −0.1, 0, 0.1, and 0.2 per Å upwardly. For a given value of the curvature modulus κ_{cv}, the surface tension decreases as β increases. The gating energy can be negative with a negative spontaneous curvature of the inner layer even though the surface tension is relatively weak.

$$u_s(r) = A_+K_0(k_+r) + A_-K_0(k_-r), \quad (3)$$

$$k_\pm = \sqrt{\frac{1}{2}\left(\frac{1}{\beta} \pm \sqrt{\frac{1}{\beta^2} - \frac{4\alpha}{\beta h_0^2}}\right)}. \quad (4)$$

For a real solution $\alpha\beta \leqslant (h_0/2)^2$ and $A_\pm$ can be determined by boundary conditions at the interface r_0, such as $u_s(r_0) \equiv u_0$ and $(du_s/dr)|_{r_0} \equiv u_0'$.

To determine the contact position of the membrane to the channel and u_0 of each layer, we assume a strong hydrophobic interaction between the channel proteins and the nearby lipid molecules. This assumption makes the membrane deform to eliminate the hydrophobic mismatch at the interface completely. Hence the contact positions can be written as $r_0^{out,closed(open)}=a+(p_0\sin\theta)/2$, $r_0^{in,closed}=a-(p_0\sin\theta)/2$ and $r_0^{in,open}=a$. *out* (*in*) denotes the outer (inner) layer, and *closed* (*open*) denotes the closed (open) state. θ is the inclined angle of the channel to the normal direction of membrane, a is the distance from the center of the channel pore to the hinge, and p_0 is the hydrophobic length of the channel proteins as shown in Fig. 1. It is important to note that the inner contact position changes during the gating process. The membrane perturbation at each contact position is also determined by the strong hydrophobic interaction, which eliminates the hydrophobic mismatch completely, as $u_0^{out,closed(open)}=(p_0\cos\theta - h_0)/2$, $u_0^{in,closed}=-(p_0\cos\theta - h_0)/2$, and $u_0^{in,open}=-(p_0 - h_0)/2$.

The last boundary condition u_0' describing the contact slope of the perturbed membrane is not determined by the strong hydrophobic boundary condition. However, we can choose u_0' as the one which minimizes the deformation free energy $F(u_0')$ if any molecular details at the contact boundary are neglected [12].

We now define a gating energy as $\Delta f=f_{open}-f_{closed}$, where $f_{open(closed)}=F^{out}_{open(closed)}+F^{in}_{open(closed)}$. $F^{out(in)}_{open(closed)}$ denotes the

deformation energy of the outer (inner) layer in the open (closed) conformation. According to the definition, the channel is in the open state when the gating energy is negative. Hence we investigate the transition of the gating energy from positive to negative. By inserting $u_s(r)$ into Eq. (1), it is straightforward to express the deformation free energy F as combinations of the modified Bessel functions of the second kind, K_n,

$$\frac{F}{\kappa_{st}} = \frac{\pi A_+^2 r_0^2}{2}\left[\left(\frac{\alpha}{h_0^2}+\beta k_+^4\right)[K_1^2(k_+r_0)-K_0^2(k_+r_0)]+k_+^2[K_0(k_+r_0)K_2(k_+r_0)-K_1^2(k_+r_0)]\right]+\frac{\pi A_-^2 r_0^2}{2}\left[\left(\frac{\alpha}{h_0^2}+\beta k_-^4\right)[K_1^2(k_-r_0)-K_0^2(k_-r_0)]\right.$$
$$\left.+k_-^2[K_0(k_-r_0)K_2(k_-r_0)-K_1^2(k_-r_0)]\right]-\frac{2\pi A_+A_-k_+r_0}{k_+^2-k_-^2}\left(\frac{\alpha}{h_0^2}+\beta k_+^2k_-^2-k_-^2\right)K_1(k_+r_0)K_0(k_-r_0)+\frac{2\pi A_+A_-k_-r_0}{k_+^2-k_-^2}\left(\frac{\alpha}{h_0^2}+\beta k_+^2k_-^2\right.$$
$$\left.-k_+^2\right)K_1(k_-r_0)K_0(k_+r_0)-2\pi\beta C_0[A_+k_+r_0K_1(k_+r_0)+A_-k_-r_0K_1(k_-r_0)]. \quad (5)$$

We would like to emphasize again that $k_\pm$ depends only on the intrinsic physical properties of the membrane such as moduli and thickness. On the other hand, $A_\pm$ is strongly related to the contact position and the boundary conditions at the interface—which are r_0, u_0, and u_0'. Fortunately the gating energy of our system is simplified as

$$\Delta f = F_{open}^{in} - F_{closed}^{in}, \quad (6)$$

since only the deformation of the inner changes during the transition from the conical conformation to the bottleneck conformation. In other words, $F_{open}^{out}=F_{closed}^{out}$. For the same reason we introduce the spontaneous curvature C_0 only into the inner layer.

To evaluate the gating energy Δf quantitatively, we collect some biological data from previously reported works [1,2,11] such as $h_0=35$ Å, $a=20$ Å, and $\theta=30°$. We choose 50 Å as a hydrophobic length p_0 of the channel protein to impose a sufficient hydrophobic mismatch regardless of the channel conformation. For simplicity we first neglect the thickness change of the membrane; hence, $\alpha=0$. It is natural to normalize the gating energy to κ_{cv} when $\alpha=0$ since $\beta=\kappa_{cv}/\kappa_{st}$ in Eq. (5). $\Delta f/\kappa_{cv}$ as a function of $\log_{10}(\beta/\text{Å}^2)$ is plotted in Fig. 2 for several values of the spontaneous curvature of the inner layer. Remarkably, it shows that the gating energy is always positive for zero or positive spontaneous curvature and can be negative as long as $C_0<0$. This means that the channel cannot be in the open state even with a quite strong surface tension if we do not impose a negative spontaneous curvature to the inner layer under the influence of the hydrophobic interaction. On the other hand, the negative spontaneous curvature can induce a channel opening even with relatively weak surface tension. The importance of the hydrophobic interaction can be understood easily by recalling the work of Ref. [2] in which the hydrophobic interaction was not considered in any manner and the open-channel state is stable at $C_0=0$ as long as the surface tension of the membrane is strong enough. When negative spontaneous curvature is introduced, the open state is stable for a quite large range of membrane tension from 10^{-14} N/Å to 10^{-11} N/Å if $\kappa_{cv}\sim 10^{-9}$ N Å (or $25k_BT$ equivalently). The open state can be also favorable even for a very weak $\kappa_{cv}(\sim 0.25k_BT)$ as long as the membrane has a large tension. For instance, the negative gating energy becomes a few k_BT when $\kappa_{st}\sim 10^{-13}$ N/Å if $C_0\sim -0.1/\text{Å}$.

The correlation of the surface tension and the spontaneous curvature in the gating energy can be seen more easily by plotting $\Delta f/\kappa_{cv}$ as a function of C_0 as shown in Fig. 3. The spontaneous curvature needed to open the channel decreases significantly as β increases. The critical values of the spontaneous curvature at which $\Delta f=0$ are -4.3×10^{-2}/Å, -4.5×10^{-3}/Å, -4.7×10^{-4}/Å, and -4.9×10^{-5}/Å, respectively, when $\beta/\text{Å}^2=10^2$, 10^3, 10^4, and 10^5. It suggests that the channel can be in the open state at a sufficiently weak membrane tension if a little bit of the spontaneous curvature is considered. For instance, when $\log_{10}(\beta/\text{Å}^2)=5$ in which $\kappa_{st}\sim 10^{-14}$ N/Å (or 10^{-15} N/Å) if $\kappa_{cv}\sim 25k_BT$ (or $2.5k_BT$), the gating energy becomes negative as long as $C_0<-4.9\times10^{-5}$/Å.

Now we consider the effect of thickness change of the membrane on the gating energy. For this purpose we assume that $\alpha=10$; hence, $\kappa_{th}=10\kappa_{st}$. When $\alpha\neq 0$, it is more conve-

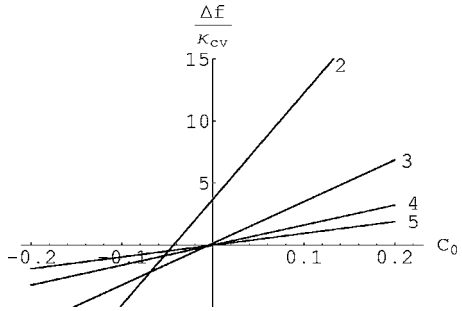

FIG. 3. The gating energy $\Delta f/\kappa_{cv}$ as a function of C_0 when $\alpha=0$. The numbers, 2, 3, 4, and 5 are four values of $\log_{10}(\beta/\text{Å}^2)$ and refer to four values of the surface tension corresponding to 10^{-11}, 10^{-12}, 10^{-13}, and 10^{-14} in N/Å, respectively, when $\kappa_{cv}=25k_BT$. The gating energies become negative if the spontaneous curvatures are less than -4.3×10^{-2}, -4.5×10^{-3}, -4.7×10^{-4}, and -4.9×10^{-5} per Å for $\log_{10}(\beta/\text{Å}^2)=2$, 3, 4, and 5, respectively.

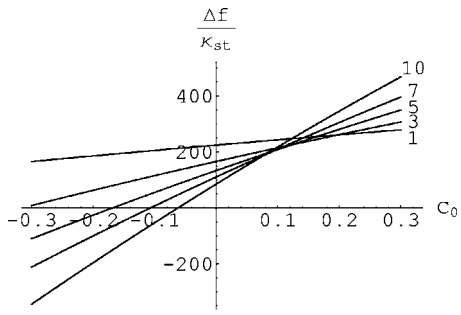

FIG. 4. The gating energy $\Delta f/\kappa_{st}$ as a function of C_0 when $\alpha=10$. The numbers are five values of $\beta/\text{Å}^2$. The spontaneous curvatures at which $\Delta f=0$ are −1.0, −0.32, −0.17, −0.11, and −0.06 per Å, respectively, for increasing order of β.

nient to plot $\Delta f/\kappa_{st}$ instead of $\Delta f/\kappa_{cv}$. We plot $\Delta f/\kappa_{st}$ in Fig. 4 as a function of C_0 for five values of β corresponding to 1, 3, 5, 7, and 10 in Å^2. According to the fact that at least a few k_BT of $|\Delta f|$ are needed for a stable open state, we also restrict the range of the curvature modulus from 10^{-11} N Å to 10^{-10} N Å. Hence for $\kappa_{cv}=10^{-11}$ N Å, it covers κ_{th} from 10^{-11} N/Å to 10^{-10} N/Å and κ_{st} from 10^{-12} N/Å ($\sim 0.025 k_BT/\text{Å}^2$) to 10^{-11} N/Å ($\sim 0.25 k_BT/\text{Å}^2$). On the other hand, for $\kappa_{cv}=10^{-10}$ N Å, it covers κ_{th} from 10^{-10} N/Å to 10^{-9} N/Å and κ_{st} from 10^{-11} N/Å($\sim 0.25 k_BT/\text{Å}^2$) to 10^{-10} N/Å($\sim 2.5 k_BT/\text{Å}^2$). The spontaneous curvatures at which the channel starts to open are −1.0, −0.32, −0.17, −0.11, and −0.06 per Å, respectively, for increasing order of β. Taking into account of small biological values for C_0, the most reasonable value of β is 10 Å^2 among those. When $\beta=10$ Å^2 and κ_{st} ranges from $0.025 k_BT/\text{Å}^2$ to $0.25 k_BT/\text{Å}^2$, $\Delta f/\kappa_{st}$ reduces to values in which the channel can be in open states. It implies that not only a negative spontaneous curvature but also a relatively strong surface tension is required to open the channel if the membrane thickness responds sensitively to the conformational change of the channel proteins.

In Table I, we summarize some plausible sets of values for the physical moduli needed to open the channel. These sets are not complete since we analyzed the gating energy in terms of ratios of the moduli. Hence there could be more sets of moduli describing real biological membranes well. These theoretical values of moduli for the transition from the conical closed state to the bottleneck open state might provide some references to experiments related to gating mechanisms of ion channels although our model is not complete.

Membrane deformation possibly induced by various stimuli can be strongly related to the gating mechanisms of ion channels. In this paper, we regarded the membrane as two effectively independent elastic planes and evaluated the deformation free energy with care, especially focusing on the gating mechanism with a hinge in the middle of the membrane. Since the channel conformations we dealt with are conical and bottlenecked, there was no structural symmetry about the midline of the membrane as usually treated in elsewhere. In our system, only the inner part of the channel changes its conformation during the gating process. For the analysis, we imposed a strong hydrophobic boundary condition at the interface between the channel and membrane lipids so that the local undulation at the interface was frustrated to eliminate the hydrophobic mismatch between them. Under the presence of the hydrophobic interaction, a role of the spontaneous curvature was crucial to open the channel. Introducing a spontaneous curvature of monolayer is equivalent to a difference of lipid concentrations between the outer layer and the inner layer of the membrane. Perozo *et al.* [7] observed that an ion channel can easily open in the presence of a lipid concentration difference even for a very weak surface tension. Our result was consistent with this observation. The effect of spontaneous curvature on the gating process will be more important when the hydrophobic mismatch is small, in the sense that the open state can be stable at a very moderate spontaneous curvature. It was also pointed out that surface tension could play a significant role even in the presence of a hydrophobic interaction if the energy cost for the thickness change of the membrane could not be neglected even though a negative spontaneous curvature is still required to open the channel. We listed some values of physical moduli describing the elastic membrane on which the channel could be in stable open states. We expect these values to provide some guidelines for related experiments. We could generalize this work by including a conformational change of the upper part of the channel. The simplest generalization will be a gating mechanism with a pivot in the middle of the membrane; hence, the channel proteins rotate as a whole with respect to the pivot. We will discuss this generalization elsewhere.

TABLE I. Sets of physical moduli needed for negative gating energies. For each set the magnitude of gating energy ranges from a few k_BT to a few hundreds of k_BT.

	κ_{th} (N/Å)	κ_{cv} (N Å)	κ_{st} (N/Å)	C_0 (/Å)
1	0	10^{-9}	$10^{-11}-10^{-14}$	$<-5\frac{\kappa_{st}}{\kappa_{cv}}$ [a]
2	0	10^{-10}	$10^{-12}-10^{-15}$	
3	10^{-11}	10^{-11}	10^{-12}	<-0.06
4	10^{-10}	10^{-10}	10^{-11}	

[a]Here values from 4.3 to 4.9 in Fig. 3 are approximated to 5 without any loss of gating properties.

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