# DEFORMATION FREE ENERGY OF BILAYER MEMBRANE AND ITS EFFECT ON GRAMICIDIN CHANNEL LIFETIME

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ABSTRACT The deformation free energy of a lipid bilayer is presented based on the principle of a continuum theory. For small deformations, the free energy consists of a layer-compression term, a splay-distortion term, and a surface-tension term, equivalent to the elastic free energy of a two-layer smectic liquid crystal with surface tension. Minimization of the free energy leads to a differential equation that, with boundary conditions, determines the elastic deformation of a bilayer membrane. When a dimeric gramicidin channel is formed in a membrane of thickness greater than the length of the channel, the membrane deformation reduces the stability of the channel. Previously this effect was studied by comparing the variation of channel lifetime with the surface tension of bilayers (Elliott, J. R., D. Needham, J. P. Dilger, and D. A. Hayden, 1983, *Biochim. Biophys. Acta,* 735:95–103). The tension was assumed to pull a dimer for a distance $z$ before the channel loses ion conductivity. To account for the data, $z$ was found to be 18 Å. With the deformation free energy, the data can be accounted for with $z \lesssim 1$ Å, which is consistent with the breaking of hydrogen bonds in a dimer dissociation. Increasing the strength of lipid–protein interactions is not the only consequence of the complete free energy compared with the previous discussions. It also changes the shape of membrane deformation around an embedded channel from convex to concave, and increases the range of deformation from <10 Å to >20 Å. Clearly these will be important factors in the general considerations of lipid–protein interactions and membrane-mediated interactions between proteins. In addition, thermal fluctuations of a membrane are calculated; in particular, we calculate the relations between the intrinsic thickness and the experimentally measured values. The experimental parameters of monoolein-squalene membranes are used for quantitative analyses.

## INTRODUCTION

The elastic properties of lipid bilayer membranes that are regarded as continuous media have been studied in many different contexts. These studies ranged from local phenomena, such as lipid–protein interactions (Abney and Owicki, 1985, and references cited therein), to the shape fluctuations (the flicker phenomenon) of whole cells (Brochard and Lennon, 1975). During the last decade or so, ingenious techniques were developed to measure the basic elastic constants of membranes, such as the thickness compressibility, the surface tension coefficient, the lateral compressibility, and the curvature elastic modulus (Evans and Skalak, 1980; Hladky and Gruen, 1982; Lis et al., 1982; Needham and Haydon, 1983; Schneider et al., 1984; Engelhardt et al., 1985). In this paper we will use the measured elastic constants to consider the deformation free energy of a membrane whose minimum-energy configuration is planar and whose circumferential boundary is fixed, like a black lipid membrane.

Recently this problem of deformation free energy was discussed in connection with the membrane thickness fluctuations (Hladky and Gruen, 1982, 1984) and with the effect of membrane thickness on the lifetime of gramicidin channel (Elliott et al., 1983). Two types of deformations were considered in previous discussions, i.e., the change in thickness and the change in surface area, which respectively give rise to a compression term and a surface tension term in the free energy. Here we will discuss a more complete deformation free energy, including a term corresponding to the splay distortion in liquid crystals. The splay term is important—in fact, it dominates the free energy—for deformations of wavelength close to the membrane thickness. Therefore, it must be considered in the thickness effect of gramicidin channel lifetime and similar membrane–protein interactions. It will also be shown that the splay term controls the wrinkling fluctuations and the breathing-model type fluctuations proposed by Miller (1984). Consequently both the thickness fluctuations and the surface expansion fluctuations are small.

For definitiveness, we will consider only homogeneous, solventless lipid bilayer membranes. (The structure, i.e., the molecular distribution, of a solvent-containing membrane has not been determined experimentally; see, for example, Gruen [1981] and Gruen and Hladky [1981]). In Section II, we derive the deformation free energy. For deformations of wavelength longer than the layer thickness, the free energy consists of three terms, i.e., a layer-compression term, a surface tension term and a splay term. Although each of these terms has been discussed before

(Kramer, 1971; Helfrich, 1973; Evans and Skalak, 1980; Hladky and Gruen, 1982, 1984), we feel that it is worthwhile to briefly go through the essential steps of deriving the deformation free energy from the principle of elasticity (Landau and Lifshitz, 1970) so as to ascertain that the terms kept are necessary and sufficient. Essentially, the free energy density is derived by expanding in powers of spatial derivatives of deformation displacements. The types of terms allowed in the free energy density are restricted by the symmetry of the system (Landau and Lifshitz, 1969, Chap. XIII). Since the symmetry property of a lipid bilayer is similar to a smectic liquid crystal, and the elastic theory of liquid crystals has been thoroughly studied (de Gennes, 1974; Stephen and Straley, 1974), the arguments used for liquid crystals can be used to restrict the form of the free energy for membrane. We then use the well studied glyceryl monooleate membranes with squalene as an example to compare the relative importance of individual terms in the free energy. For wavelength >180 Å, we found compression > tension > splay; for 50 Å < wavelength < 180Å, compression > slay > tension; and for wavelength <50 Å, splay > compression > tension. (The thickness of the hydrocarbon core of a glyceryl monooleate/squalene bilayer is 25 Å.) In Section III, we calculate the fluctuations in membrane thickness and surface area. (The key steps of the computations are given in the Appendix.) In particular we calculate the relations between the intrinsic thickness and the average thickness as determined by electrical capacitance or optical reflectance measurements. The discrepancy between the root-mean-square thickness (by reflectance) and the mean reciprocal thickness (by capacitance) is <1%.

In Section IV the thickness effect on gramicidin channel lifetime is discussed. When dimeric gramicidin channels are formed in membranes of thickness greater than the length of the channel, it was found that the measured channel lifetime decreases with the membrane thickness. It is reasonable to assume that a thick membrane deforms locally to accommodate a channel, and that the force resulting from the deformation reduces the stability of the dimeric formation. Elliott et al. (1983) assumed that the force is mainly the surface tension and the work done by the tension over an unknown distance $z$ represents the reduction of the activation energy for the dimer dissociation. However, to account for the experimental data, $z$ would have to be 18 Å. That would imply that membrane tension pulls a dimer apart over a distance of 18 Å before the dimer loses (or significantly changes) its ion conductivity. A more plausible explanation of the thickness effect is found by using the more complete free energy. We will show that the local deformations under consideration involve wavelengths close to the bilayer thickness, so that the deformation free energy is dominated by splay and compression. We find that, with the splay and compression terms, the data of Elliott et al. (1983) can be explained with $z \lesssim 1$ Å. As a consequence, we are also able to determine the shape of the membrane deformation resulting from a channel formation; this is discussed in Section V.

## DEFORMATION FREE ENERGY

As mentioned in the introduction, a brief review of the theory of smectic liquid crystals will facilitate our discussion. Consider a smectic A in which elongated molecules are arranged in layers and the preferred direction of the long axes of molecules is perpendicular to the plane of the layers. In the state of minimum energy, the layers are equidistant, all parallel to one plane, and the long axes of molecules in the preferred direction. Let the $z$-axis be the normal to the plane. Now consider some small, long wavelength distortions imposed on this initial state. Let $u_n(x, y)$ be the displacement of the $n$th layer in the $z$-direction. Strictly speaking, the molecular axes do not remain exactly normal to the new plane of the layers. However, it can be shown that the deviations of the preferred direction from the normal to the plane require relatively large energies (de Gennes, 1972). Thus, for small distortions, we may assume that, at each point, the molecular axes are normal to the layers. Regarding the system as a continuous medium, one replaces $u_n(x, y)$ by $u(x, y, z)$. The distortion free energy $f$ (per unit volume of the unperturbed system) can be shown to have the following form (de Gennes, 1969):

$$f = f_0 + \frac{1}{2}\bar{B}\left(\frac{\partial u}{\partial z}\right)^2 + \frac{1}{2}K_1\left(\frac{\partial^2 u}{\partial x^2} + \frac{\partial^2 u}{\partial y^2}\right)^2. \qquad \text{(II.1)}$$

$f_0$ is the unperturbed free energy. The second term represents an elastic energy for compression of the layers. The third term represents the energy of splay distortion (de Gennes, 1974). Two other possible distortions in nematic liquid crystals, i.e., twist and bend, are unfavorable in smectics. Other terms that are of higher order in space variations of $u$ have also been neglected. Thus, for small, long-wavelength distortions, the static elastic properties of a smectic $A$ are described by two constants: $\bar{B}$ (dimension energy length$^{-3}$) and $K_1$ (dimension energy length$^{-1}$). The associated length

$$\xi = (K_1/\bar{B})^{1/2} \qquad \text{(II.2)}$$

should be comparable to the layer thickness. $K_1$ is typically about $10^{-6}$ dynes in room temperature (de Gennes, 1974; Stephen and Straley, 1974).

For the deformation free energy of a membrane, we must add a surface tension term to Eq. II.1. If $\gamma$ is the surface tension of a surface layer whose displacement in the $z$-direction is $u(x, y)$, then the free energy increase per unit area due to the surface area change from its planar

ate is given by

$$\left\{\left[1+\left(\frac{\partial u}{\partial x}\right)^2+\left(\frac{\partial u}{\partial y}\right)^2\right]^{1/2}-1\right\} \approx \frac{\gamma}{2}\left[\left(\frac{\partial u}{\partial x}\right)^2+\left(\frac{\partial u}{\partial y}\right)^2\right], \quad \text{(II.3)}$$

here the approximation is consistent with the derivation Eq. II.1.

To describe a bilayer membrane, let the equilibrium onfiguration be parallel to the $x$-$y$ plane. Let $u_+(x, y)$ and $_-(x, y)$ be the displacements of the top and bottom irfaces from their equilibrium positions, respectively. The quilibrium half thickness of the bilayer is denoted by $a$. ollowing Eqs. II.1 and II.3, we have the deformation free ıergy of a bilayer membrane, per unit area of the nperturbed system,

$$F = F_0 + \frac{2a}{2}\bar{B}\left(\frac{u_+ - u_-}{2a}\right)^2 + \frac{a}{2}K_1\left[\left(\frac{\partial^2 u_+}{\partial x^2}+\frac{\partial^2 u_+}{\partial y^2}\right)^2+\left(\frac{\partial^2 u_-}{\partial x^2}+\frac{\partial^2 u_-}{\partial y^2}\right)^2\right] + \frac{\gamma}{2}\left[\left(\frac{\partial u_+}{\partial x}\right)^2+\left(\frac{\partial u_+}{\partial y}\right)^2+\left(\frac{\partial u_-}{\partial x}\right)^2+\left(\frac{\partial u_-}{\partial y}\right)^2\right]. \quad \text{(II.4)}$$

$_0$ is the unperturbed free energy. The other three terms re, respectively, the layer-compression, splay, and tension nergies. From $u_+$ and $u_-$, we can construct two indepenent modes $D$ and $S$:

$$D(x, y) = u_+(x, y) - u_-(x, y), \quad \text{(II.5)}$$

$$S(x, y) = u_+(x, y) + u_-(x, y). \quad \text{(II.6)}$$

ı terms of $D$ and $S$, the free energy becomes

$$F = F_0 + a\bar{B}\left(\frac{D}{2a}\right)^2 + \frac{a}{4}K_1\left[\left(\frac{\partial^2 D}{\partial x^2}+\frac{\partial^2 D}{\partial y^2}\right)^2+\left(\frac{\partial^2 S}{\partial x^2}+\frac{\partial^2 S}{\partial y^2}\right)^2\right] + \frac{\gamma}{4}\left[\left(\frac{\partial D}{\partial x}\right)^2+\left(\frac{\partial D}{\partial y}\right)^2+\left(\frac{\partial S}{\partial x}\right)^2+\left(\frac{\partial S}{\partial y}\right)^2\right]. \quad \text{(II.7)}$$

For both the thickness fluctuations and the thickness ffect on gramicidin, we will be interested in the $D$-modes. onsider a square membrane with the length of each side . Define the $D$-mode free energy $\mathcal{F}_D = \int F_D\, dx\, dy$, where $_D$ represents the $D$-mode terms in Eq. II.7 and the ıtegration is over the squre $L^2$. Decomposing $D(x, y)$ into Fourier series

$$D(\mathbf{r}) = \sum_{\mathbf{k}} D_{\mathbf{k}}\, e^{i\mathbf{k}\cdot\mathbf{r}}, \quad \text{(II.8)}$$

we obtain

$$\mathcal{F}_D = \frac{L^2}{4}\sum_{\mathbf{k}} D_{\mathbf{k}} D_{-\mathbf{k}}\left[\frac{\bar{B}}{a}+\gamma k^2 + aK_1 k^4\right]. \quad \text{(II.9)}$$

The experimental data for the parameters, except $K_1$, are available for glyceryl monooleate membranes with squalene. The total thickness of the hydrocarbon core of a bilayer is $2a$ = 25.0 Å (White, 1978; Dilger, 1981). The compressibility coefficient $\bar{B}$ is 5.0 × $10^{-8}$ dynes Å$^{-2}$ (Hladky and Gruen [1982] obtained the value of $\bar{B}/[2a]$ from the measurement of White [1978]). The half-bilayer tension $\gamma$ is 1.5 × $10^{-8}$ dynes Å$^{-1}$ (Hladky and Gruen, 1982; see this reference's Appendix III).

The splay coefficient $K_1$ is related to the curvature elastic modulus $K_c$ by $K_c = 2aK_1$ (Helfrich, 1973; Schneider et al., 1984). Two sets of values have been reported for $K_c$ of lecithin vesicles and red blood cells: a high $K_c$ = 1–2 × $10^{-12}$ erg and a low $K_c$ = 2–7 × $10^{-13}$ erg (for the details see the following reference). Recently Engelhardt et al. (1985) were able to explain this discrepancy and concluded that the lower values are the correct ones. Further, since the measured $K_c$ is proportional to the total thickness of the vesicle wall, and since the number of bilayers forming the vesicle wall was uncertain, the authors suggested that the lower values of their measurements $K_c$ = 2.8–6.5 × $10^{-13}$ erg are closer to the true value. Using $2a \approx$ 30 Å for lecithin bilayers (Büldt et al., 1978), one obtains $K_1 \approx 10^{-6}$ dynes for membranes, same as in liquid crystals as mentioned above. In the following discussions we shall use this value for $K_1$.

First we will compare the contributions of compression, splay, and tension to the deformation free energy as a function of wavelength. This is accomplished by comparing the magnitudes of the three terms in Eq. II.9 with $k = 2\pi/\lambda$. The result is shown in Table I. Hladky and Gruen (1982) correctly argued that long-wavelength ($\lambda \gg a$) deformations are adequately described by compression and tension. They also pointed out that in deformations of wavelength close to the layer thickness, the crowding of the lipid chains may occur. The latter is now taken into account by the splay term. As long as a membrane can be regarded as a continuous medium, the mechanics of its small deformations can be described by the free energy (Eq. II.7). Here, small deformations mean small gradients in $u_+$ and $u_-$; physically, this means that the membrane surfaces are not very much tilted from the $x$-$y$ plane. This

TABLE I
COMPARISON OF LAYER-COMPRESSION, SPLAY, AND SURFACE TENSION ENERGIES AS A FUNCTION OF WAVELENGTH

| | |
|---|---|
| 50 Å > λ > 10 Å | Splay > compression > tension |
| 180 Å > λ > 50 Å | Compression > splay > tension |
| λ > 180 Å | Compression > tension > splay |

restriction excludes certain interesting cases, such as the folding of a membrane.

## THICKNESS AND AREA FLUCTUATIONS

Since a membrane is a deformable body, it is important to understand the relation between its intrinsic thickness and the experimentally measured values. The standard method of determining the thickness of a lipid bilayer is the electrical capacitance measurement (Hanai et al., 1964). The measured value of capacitance per unit area is proportional to the mean reciprocal thickness $\langle 1/h \rangle$, where $h$ is the thickness of the hydrocarbon region of the membrane. According to the notations in Section II, we have

$$h = 2a + D \tag{III.1}$$

and, for small fluctuations,

$$\left\langle \frac{1}{h} \right\rangle = \frac{1}{2a}\left[1 - \frac{\langle D \rangle}{2a} + \frac{\langle D^2 \rangle}{(2a)^2} - \frac{\langle D^3 \rangle}{(2a)^3} + \frac{\langle D^4 \rangle}{(2a)^4} + \cdots\right]. \tag{III.2}$$

The brackets $\langle\ \rangle$ represent the ensemble averages that can be calculated by the standard fluctuation theory (e.g., Landau and Lifshitz, 1969, Chap. XII). It can be shown that (see Appendix)

$$\langle 1/h \rangle^{-1}/(2a) = 1 - [\langle D^2 \rangle/(2a)^2 + 2\langle D^2 \rangle^2/(2a)^4 + \cdots] \tag{III.3}$$

with

$$\langle D^2 \rangle = \frac{k_B T}{2\pi a K_1 C_2}\left\{\tan^{-1}\left[\left(\left(\frac{2\pi}{\lambda_0}\right)^2 + C_1\right)\Big/ C_2\right] - \tan^{-1}(C_1/C_2)\right\}, \tag{III.4}$$

$$C_1 = \frac{\gamma}{2aK_1} \sim 6.0 \times 10^{-4}\ \text{Å}^{-2}, \tag{III.5}$$

$$C_2 = \left(\frac{\bar{B}}{a^2 K_1}\right)^{1/2} \sim 1.8 \times 10^{-2}\ \text{Å}^{-2}, \tag{III.6}$$

where $k_B$ is the Boltzmann constant and $T$ the temperature. $\lambda_0$ represents the cutoff wavelength, which is approximately the layer thickness $a$. In other words, an ensemble average is the average over the spontaneous thermal deformations with wavelength ranging from infinite to $\lambda_0$. Deformations of wavelength shorter than $a$ are presumably suppressed by higher order (in spatial derivatives) terms in free energy. For the thermal fluctuations and the type of deformations considered in the next section, these higher order terms have negligible contributions. Therefore they are not included in the free energy Eq. II.4.

Table II shows the root-mean-square (rms) fluctuations in thickness $\langle D^2 \rangle^{1/2}$ in comparison with the calculations without the splay term ($K_1 = 0$) (Hladky and Gruen, 1982). We note that the presence of the splay term greatly reduces the contribution of the fluctuations in the range $\lambda = 100$ Å to $\lambda = 10$ Å. The thickness determined from capacitance $\langle h \rangle^{-1}$ is smaller than the intransic thickness $2a$ by a fraction shown in the bracket in Eq. III.3. The numerical value is shown in Table II along with the corresponding value if $K_1 = 0$. The thickness of a lipid bilayer can also be determined from its optical reflectance (Cherry and Chapman, 1969). The latter is related to the mean square of the total membrane thickness, including both the polar and hydrocarbon regions. For the purpose of numerical comparison with the capacitance measurement, we compute the mean square of the hydrocarbon thickness $\langle h^2 \rangle$ or

TABLE II
FLUCTUATIONS OF GLYCERYL MONOOLEATE-SQUALENE MEMBRANES ($T$ = 300K), WITH OR WITHOUT ($K_1$ = 0) SPLAY ENERGY

| Cutoff wavelength $\lambda_0$ | 100 Å | 10 Å |
|---|---|---|
| rms Fluctuations in thickness | | |
| $\langle D^2 \rangle^{1/2}$ | 0.81 Å | 2.1 Å |
| $\langle D^2 \rangle^{1/2}_{K_1=0}$ | 0.82 Å | 6.4 Å |
| Capacitance measurement | | |
| $\langle 1/h \rangle^{-1}/2a$ | | 0.993 |
| $(\langle 1/h \rangle^{-1}/2a)_{K_1=0}$ | | 0.94 |
| Reflectance measurement | | |
| $\langle h^2 \rangle^{1/2}/2a$ | | 1.004 |
| $(\langle h^2 \rangle^{1/2}/2a)_{K_1=0}$ | | 1.03 |
| Fractional area expansion | | |
| $\langle \Delta A \rangle/2L^2$ | | 5.9% |
| $\langle \Delta A \rangle_{K_1=0}/2L^2$ | | 304% |

$$\langle h^2 \rangle^{1/2}/2a = [1 + \langle D^2 \rangle/(2a)^2]^{1/2} \tag{III.7}$$

and show the results in Table II. We see that thermal fluctuations cause no appreciable difference between different averages.

Unlike the thickness fluctuations in which the average of the first-order fluctuation $\langle D \rangle$ vanishes, the fluctuations in surface area are always positive—all deformations increase the surface area. The mean fractional increase in area is given by

$$\frac{1}{2}\frac{\langle \Delta A \rangle}{L^2} = \frac{1}{8L^2}\int dx\, dy \left\langle \left(\frac{\partial S}{\partial x}\right)^2 + \left(\frac{\partial S}{\partial y}\right)^2 + \left(\frac{\partial D}{\partial x}\right)^2 + \left(\frac{\partial D}{\partial y}\right)^2 \right\rangle \tag{III.8}$$

according to Eq. II.3. The factor ½ is because $\Delta A$ includes two (top and bottom) surfaces. The calculation of $\langle \Delta A \rangle$ is similar to that for $\langle D^2 \rangle$, except that the former contains both the $S$- and $D$-modes. It is instructive to examine the integrals for $\langle \Delta A \rangle$:

$$\frac{1}{2}\frac{\langle \Delta A \rangle}{L^2} = \frac{k_B T}{16\pi}\int_0^{(2\pi/\lambda_0)^2} dk^2 \left\{\frac{1}{aK_1 k^2 + \gamma} + \frac{k^2}{aK_1 k^4 + \gamma k^2 + \bar{B}/a}\right\}, \tag{III.9}$$

where the first and second integrals are, respectively, the $S$- and $D$-mode contributions. Here it is easy to see how the splay term suppresses fluctuations. Without the splay term ($K_1 = 0$), the $S$-mode integral diverges linearly as $(2\pi/\lambda_0)^2$. Similarly, with $K_1 = 0$, the $D$-mode integral contains a term linearly divergent. That means the short-wavelength $S$-modes (wrinkling) and the short-wavelength $D$-modes (the breathing model proposed by Miller [1984]) would dominate the fluctuations. And the resulting mean increase in area would be three times the equilibrium value (Hladky and Gruen, 1982). In reality, with the presence of the splay term, the integrals in Eq. III.9 are logarithmic:

$$\frac{1}{2}\frac{\langle \Delta A \rangle}{L^2} = \frac{k_B T}{16\pi a K_1}\left\{\ln\left(\frac{\left(\frac{2\pi}{\lambda_0}\right)^2 + 2C_1}{2C_1}\right) + \frac{1}{2}\ln\left(\frac{\left[\left(\frac{2\pi}{\lambda_0}\right)^2 + C_1\right]^2 + C_2^2}{C_1^2 + C_2^2}\right) - \frac{C_1}{C_2}\left[\tan^{-1}\left(\frac{\left(\frac{2\pi}{\lambda_0}\right)^2 + C_1}{C_2}\right) - \tan^{-1}\left(\frac{C_1}{C_2}\right)\right]\right\} \quad \text{(III.10)}$$

and the mean increase in area is only 6% of the equilibrium value (Table II).

## EFFECT OF BILAYER THICKNESS ON GRAMICIDIN CHANNEL LIFETIME

It is now well accepted that ion conduction mediated by gramicidin in bilayer membrane occurs by pore formation and the pores are dimers of the polypeptide, i.e., two single stranded $\beta$-helical polypeptides linked head-to-head (head ≡ formyl end) by six hydrogen bonds (Andersen, 1984; Hladky and Haydon, 1984; Urry, 1985). The kinetics of the pore (channel) formation and its dependence on membrane structure have been a subject of interest since early seventies (see the review article by Hladky and Haydon, 1984). Most experimental observations are consistent with the assumptions that the passage through the channels is the rate-limiting step for an ion crossing the membrane and that a dimeric channel loses its ion conductivity when it dissociates into monomeric units. Studies of the statistics of channel opening by conductance measurements showed that the dimeric channels and monomers are in thermal equilibrium (Hladky and Haydon, 1972; Bamberg and Läuger, 1973; Zingsheim and Neher, 1974)

$$G + G \underset{k_D}{\overset{k_R}{\rightleftharpoons}} G_2, \quad \text{(IV.1)}$$

where $G$ and $G_2$ represent the gramicidin monomers and dimers, respectively. Studies of the temperature dependence of the mean channel duration yielded the activation energies of dissociation in the range of 70 to 80 kJ/mol, consistent with the notion that several hydrogen bonds have to be broken in the dissociation reaction (Hladky and Haydon, 1972; Bamberg and Läuger, 1974). Of the two rate constants, only the dissociation constant $k_D$ has been examined in a wide range of systems. To measure the rate constant of formation $k_R$, one needs to know the membrane concentration of gramicidin. So far there is no simple and precise method of determining the concentration of gramicidin in membrane. On the other hand, $k_D$ is the reciprocal of the mean channel lifetime, which is readily measurable. Many investigations, using a variety of black lipid membranes, were conducted to study the relations between $k_D$ and the membrane thickness or the membrane tension or both of them (Elliott et al., 1983; Hladky and Haydon, 1972; Haydon and Hladky, 1972; Hendry et al., 1978; Kolb and Bamberg, 1977; Neher and Eibl, 1977; Rudnev et al., 1981).

Recently Elliott, Needham, Dilger, and Haydon (1983) proposed a theoretical model for these relations (the ENDH model). The basic idea of this model is as follows: When a dimeric gramicidin channel is formed in a membrane of thickness greater than the length of the channel, the membrane is presumably deformed locally to accommodate the channel. (The assumption that the hydrocarbon chains of lipids adapt to the thickness of the lipophilic core of a protein in the membrane has been used in other theories on protein-lipid interactions (Owicki et al., 1978; Owicki and McConnell, 1979; Mouritsen and Bloom, 1984), but they were mainly interested in the thermodynamic phase behavior of the system and the consequence on protein distributions; they also used different methods to calculate the free energies.) Then the restoring force of the deformed membrane will reduce the stability of the dimer. The ENDH model is based on the assumption that the energy of deformation is primarily the work done against membrane surface tension. Consequently the activation energy for the dissociation of a dimer will contain a term proportional to the membrane tension. According to the principle of thermodynamics, the dissociation constant $k_D$ can be written in the following form

$$k_D = \nu e^{-G^*/k_B T}, \quad \text{(IV.2)}$$

where $G^*$ is the free energy of activation and $\nu$ is a frequency factor relatively independent of temperature. As mentioned above, $G^*$ is presumably the energy required to break several hydrogen bonds linking two monomers. If the membrane tension is pulling against the hydrogen bonds, the activation energy is reduced by an amount

$$-\Delta G^* = z\ell_0 \gamma \cos\theta, \quad \text{(IV.3)}$$

where $\gamma$ is the half-bilayer tension, $\theta$ the angle between the membrane surface, and the channel axis, $\ell_0 = 2\pi r_0$ with $r_0$ being the radius of the gramicidin molecule, which is modeled as a cylinder. $z$ is the distance over which the

tension pulls before the dimer dissociates. If the model makes any sense at all, $z$ should be of the order of 1 Å or less (see below). Under this restriction, the measured values of membrane tension appeared to be at least one order of magnitude too small to account for the variation of $k_D$ with membrane tension. (In their report, ENDH used the measured values of $k_D$ and $\gamma$ to find $z \sim 18$ Å. This is to be compared with the lipophilic length of the channel ~22 Å and the bilayer thickness ~22–28 Å. They suggested the possibility that water transiently fills the gap between two parted monomers and that such water filling states maintain the channel conductivity. If this were true, it is hard to imagine that the equilibrium state would not be a distribution of channels with various degrees of water bridging. This would then be in contradiction with the exponential single-channel lifetime distribution so elegantly demonstrated by ENDH themselves and also with the reported activation energies of channel dissociation mentioned above.)

In the following we will see that the dimpling deformation taking place in a bilayer, in accommodating a channel shorter than its thickness, involves the entire range of wavelengths. Consequently, the work of deformation must include compression and splay. In fact it will be shown that the tension energy constitutes <5% of the work of deformation in this case. To proceed, consider a schematic illustration of the cross section of a bilayer with a channel embedded in the membrane as shown in Fig. 1. We shall assume that the median plane is unperturbed and the deformation is symmetric with respect to this plane. In Eq. II.4, let

$$-u_+(x, y) = u_-(x, y) = u(x, y). \tag{IV.4}$$

Then the deformation free energy per unit area simplifies to

$$F = F_0 + a\bar{B}(u/a)^2 + aK_1\left(\frac{\partial^2 u}{\partial x^2} + \frac{\partial^2 u}{\partial y^2}\right)^2 + \gamma\left[\left(\frac{\partial u}{\partial x}\right)^2 + \left(\frac{\partial u}{\partial y}\right)^2\right]. \tag{IV.5}$$

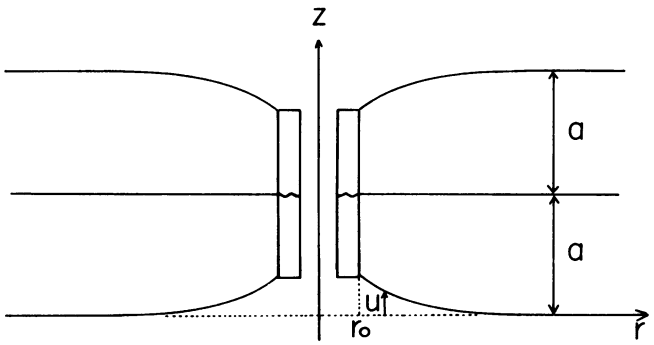


FIGURE 1 A schematic illustration of the cross-section of a bilayer with a channel embedded in the membrane.

Minimizing the free energy with respect to variations in $u(x, y)$ leads to the linear equation

$$K_1\Delta^2 u - (\gamma/a)\,\Delta u + (\bar{B}/a^2)\,u = 0 \tag{IV.6}$$

with

$$\Delta = \partial^2/\partial x^2 + \partial^2/\partial y^2. \tag{IV.7}$$

Eq. IV.6, with given boundary conditions, determines the elastic deformation of a bilayer.

The solution of Eq. IV.6 can be obtained from the solutions of the equation:

$$\Delta u = \alpha u. \tag{IV.8}$$

Substituting Eq. IV.8 in Eq. IV.6, we obtain the value of $\alpha$:

$$\alpha = \tfrac{1}{2}\{(\eta a)^{-1} \pm [(\eta a)^{-2} - 4(\xi a)^{-2}]^{1/2}\}, \tag{IV.9}$$

where $\eta$ is defined as $\eta = K_1/\gamma$ and $\xi$ is the length defined by Eq. II.2. The black-lipid membranes used in ENDH's measurement are formed from monoacylglycerols (monoeicosenoin, monoolein, monoolein plus monopalmitolein, and monopalmitolein) dissolved in squalene. These bilayers contain very little squalene (White, 1978) and will be considered solvent-free, to apply the theory that has been developed in this study. Their tension coefficients $\gamma$ were measured to be in the range of $1 \times 10^{-8}$ to $2.5 \times 10^{-8}$ dynes/Å, with the value of monoolein in the middle of the range. It is reasonable to assume that $\bar{B}$ and $K_1$ of monoolein can be used as the approximate values of $\bar{B}$ and $K_1$ for these membranes. Using $\bar{B}$, $K_1$, and $\gamma$ of monoolein given in Section II, we have

$$\eta = 67 \text{ Å}, \qquad \xi = 4.5 \text{ Å}. \tag{IV.10}$$

As a result, neglecting the tension term causes only a 3% error in $\alpha$ (Eq. IV.9). We conclude that in an elastic deformation governed by Eq. IV.8, the Fourier components of shorter wavelengths dominate the free energy. To the first-order approximation, the tension term is negligible compared with the compression and splay terms.

For mathematical simplicity, we now calculate the thickness effect on gramicidin without including the tension term. With $\gamma = 0$, the solution of Eq. IV.6 can be obtained from the solution of Eq. IV.8 with $\alpha = i/\xi a$ and the solution of Eq. IV.8 with $\alpha = -i/\xi a$. Therefore the general solution of Eq. IV.6 (with $\gamma = 0$) is

$$u = \sum_n e^{in\theta}\{A_n J_n[i^{1/2} r/(\xi a)^{1/2}] + B_n J_n[i^{3/2} r/(\xi a)^{1/2}] + C_n H_n^{(1)}[i^{1/2} r/(\xi a)^{1/2}] + D_n H_n^{(1)}[i^{3/2} r/(\xi a)^{1/2}]\} \tag{IV.11}$$

or equivalently,

$$u = \sum_n e^{in\theta}\{a_n ber_n[r/(\xi a)^{1/2}] + b_n bei_n[r/(\xi a)^{1/2}] + c_n kei_n[r/(\xi a)^{1/2}] + d_n ker_n[r/(\xi a)^{1/2}]\}, \tag{IV.12}$$

where $(r, \theta)$ are cylindrical coordinates of $(x, y)$, $J_n$ and $H_n^{(1)}$ are Bessel functions, and $ber_n$, $bei_n$, $kei_n$, and $ker_n$ are Thomson functions (Watson, 1966).

We assume that the deformations under consideration are symmetric around the $z$-axis, or $n = 0$ in Eq. IV.12, because the symmetric configuration has the lowest free energy. Also $ber(r)$ and $bei(r)$ are excluded from the solution because they are asymptotically divergent; whereas we have the boundary condition $u = 0$ as $r \to \infty$. Thus the membrane deformation induced by an embedded channel is described by

$$u = ckei\,[r/(\xi a)^{1/2}] + d\,ker\,[r/(\xi a)^{1/2}], \quad \text{(IV.13)}$$

where the indices zero are omitted by convention. The constants $c$ and $d$ are to be determined by the boundary conditions at $r = r_0$, i.e., the values of $u$ and $(\partial u/\partial r)$ at $r = r_0$, where membrane meets the channel. The outside radius of the gramicidin channel, $r_0$, can be estimated from molecular models to be approximately 10 Å (Hendry et al., 1978). The Thomson functions $kei(x)$ and $ker(x)$ are depicted in Fig. 2 (Nosova, 1961).

Briefly the experimental results of ENDH are as follows. The mean lifetime of gramicidin channels in monoacylglycerol-squalene bilayers was found to increase as the bilayer thickness decreases from 28.5 to 21.7 Å. Below 21.7 Å, the lifetime was relatively constant. Therefore, it was assumed that the length of the lipophilic exterior of the channel is ~21.7 Å. (The total length of the gramicidin channel is ~26 Å [Urry, 1985].)

We follow the idea of Eq. IV.3 that the membrane forces pull the two monomer units for a distance $z$ before the dimer loses its ion conductivity. As mentioned above, we believe that $z$ should be of order 1 Å. But even for $z = 1$ Å, one cannot assume that the membrane forces are constant during the entire process, particularly for the cases where the membranes are only a few angstroms thicker than the channel. Let $\mathcal{F}(u_0)$ be the total deformation free energy when the membrane displacement at $r_0$ is $u_0$. Instead of Eq. IV.3, we have

$$-\Delta G^* = \mathcal{F}(u_0) - \mathcal{F}\left(u_0 - \frac{z}{2}\right). \quad \text{(IV.14)}$$

Combining with Eq. IV.2, we have

$$\ln k_D(u_0) = \ln(1/\tau(u_0)) = \text{const} + (\mathcal{F}(u_0) - \mathcal{F}(u_0 - (z/2)))/k_BT, \quad \text{(IV.15)}$$

where $\tau(u_0)$ is the mean lifetime of channels embedded in a membrane of thickness

$$2a = 2u_0 + 21.7 \text{ Å}. \quad \text{(IV.16)}$$

At $2a = 21.7$ Å, $u_0 = 0$, there is no deformation. Therefore the constant in Eq. IV.15 equals to $\ln(1/\tau(0))$. Eq. IV.15, valid for $u_0 > z/2$, represents the effect of bilayer thickness on gramicidin channel lifetime.

To calculate $\mathcal{F}(u_0)$, we substitute the solution of Eq. IV.13 into the free energy density (IV.5), integrate it over the plane of membrane, and make use of the identities $\Delta kei(r) = ker(r)$ and $\Delta ker(r) = -kei(r)$:

$$\mathcal{F} = \int dxdy \left[\frac{\bar{B}}{a}u^2 + aK_1\left[\frac{\partial^2 u}{\partial x^2} + \frac{\partial^2 u}{\partial y^2}\right]^2\right] = (c^2 + d^2)(2\pi K_1/\xi)\, I[r_0/[\xi a]^{1/2}] \quad \text{(IV.17)}$$

where

$$I(x) = \int_x^\infty z dz\,[kei^2(z) + ker^2(z)] = x[kei(x)ker'(x) - ker(x)kei'(x)] \quad \text{(IV.18)}$$

(Watson, 1966). As mentioned above, constants $c$ and $d$ are supposed to be determined by the boundary conditions at $r_0$. However, the boundary condition for $\partial u/\partial r$ is unknown. Therefore we proceed as follows. We note that between $2a = 28.5$ Å ($u_0 = 3.4$ Å) and $2a = 23.0$ Å ($u_0 = 0.65$ Å), where data are available, the function $I(r_0/(\xi a)^{1/2})$ varies only slightly, from 0.17 to 0.15, compared with the change in $u_0$. Since $\mathcal{F}(u_0)$ (Eq. IV.17) should be proportional to $u_0^2$ for small $u_0$ (the same reason that the free energy density is quadratic in $u(x, y)$), $c$ and $d$ must be both approximately proportional to $u_0$:

$$c = su_0,\ d = tu_0. \quad \text{(IV.19)}$$

Between the condition $u = u_0$ at $r = r_0$ and Eq. IV.15, we have (after substituting Eq. IV.17 into Eq. IV.15) two equations for constants $s$ and $t$:

$$1 = s\,\text{kei}\,(r_0/(\xi a)^{1/2}) + t\,\text{ker}\,(r_0/(\xi a)^{1/2}), \quad \text{(IV.20)}$$

$$\ln\frac{\tau(0)}{\tau(u_0)} = (s^2 + t^2)\left(u_0 z - \frac{z^2}{4}\right)\frac{2\pi K_1}{k_B T \xi} I\left(\frac{r_0}{(\xi a)^{1/2}}\right). \quad \text{(IV.21)}$$

We note that the $u_0$-dependence of Eq. IV.21 is essentially in the factor $(u_0 z - z^2/4)$. To account for the $u_0$-dependence of $\tau(u_0)$ (Table III), one finds $z \sim 0.95$ Å. This

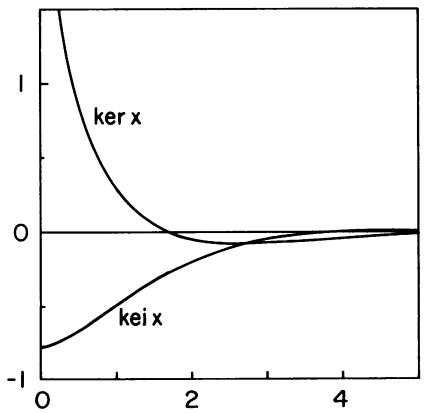


FIGURE 2 Thomson functions $kei(x)$ and $ker(x)$ (Nosova, 1961). A localized symmetric (around $z$) deformation of a membrane is described by a linear combination of $kei(x)$ and $ker(x)$.

TABLE III
THE SOLUTIONS OF EQS. IV.20 AND IV.21 FOR $s$ AND $t$ ($T$ = 296K)

| Membrane thickness $2a$* | Mean channel lifetime $\tau$* | $u_0$ | $I\left(\frac{r_0}{(\xi a)^{1/2}}\right)$ | $s$ | $t$ |
|---|---|---|---|---|---|
| Å | s | Å | | | |
| 21.7 | 286 ± 22 | 0 | | | |
| 23.0 | 119 ± 9 | 0.65 | 0.15 | −4.0 | −5.2 |
| 24.5 | 37 ± 2.5 | 1.4 | 0.15 | −3.9 | −4.3 |
| 28.5 | 0.7 ± 0.1 | 3.4 | 0.17 | −3.9 | −4.2 |

*Elliott et al. (1983).

value for $z$ is quite independent of the numerical values we used for the elastic constants $K_1$ and $\bar{B}$. Such a result makes sense if $z$ is indeed, as discussed above, an intrinsic property of the gramicidin dimer. In comparing Eq. IV.21 with experimental data, it should be noted that the factor $(s^2 + t^2)$ is not a free parameter. Rather, $s$ and $t$ have to meet the stringent requirement of satisfying the pair of Eqs. IV.20 and IV.21 for every data point. For example, we let $z$ = 1 Å and solve for $s$ and $t$ between Eqs. IV.20 and IV.21 for every data point (Table III). The agreement shown in Table III indicates that the theory is quite reasonable. Fig. 3 shows Eq. IV.21 with $s = -3.9$, $t = -4.3$ (the values of the middle point) and $z$ = 1 Å compared with experimental data. We see that the effect of bilayer thickness on gramicidin channel lifetime can be accounted for by the elastic theory of membrane.

## THE SHAPE OF DEFORMATION

The geometry of membrane in the vicinity of a protein channel may be important to the function of the channel. The shape of deformation also determines the range of perturbation induced in the plane of membrane by an embedded channel and consequently influences the lipid-mediated interactions between proteins. (Deformation is only one aspect of protein-lipid interactions. For example the lipid chains in contact with the protein surface are motionally restricted as detected by spin label ESR; see review by Marsh [1985] and other articles in the same volume. For simplicity we have neglected such interactions in the consideration of elasticity.)

Despite the lack of the complete boundary conditions at $r_0$ for the differential Eq. IV.6, we were able to determine its solution by using the gramicidin data. The deformations consistent with the variation of the channel lifetime with membrane thickness are described by

$$u(r) = -u_0\{3.9\, kei[\, r/(\xi a)^{1/2}] + 4.3\, ker[r/(\xi a)^{1/2}]\}. \quad \text{(V.1)}$$

For clarity, we plot the cross section of the bilayer that has the largest deformation ($u_0$ = 3.4 Å) in Fig. 4. It is interesting to note that near the channel the deformation is slightly concave rather than convex like Fig. 1. This is a distinct feature of the fourth order (splay) term in Eq. IV.6. Theories keeping only the second-order terms (equivalent to $K_1 = 0$ in Eq. IV.6, e.g., Owicki and McConnell, 1979) always give a convex deformation like Fig. 1, with a range of deformation about $2(\gamma a/\bar{B})^{1/2} \sim$ 7–10 Å. Remarkably, even though the slope $(\partial u/\partial r)$ at $r = r_0$ was not specified, the data and the theory require the slope to be nearly zero and the hydrocarbon chains neighboring a channel are essentially parallel to the channel. As a consequence, the surface tension at the contact is not even pulling in the direction of dissociation. Because of the concave-then-convex inflection, the range of deformation is very long, from $r$ = 10 Å to $r \sim$ 34 Å, where the displacement is ~0.1 $u_0$ (both the concave and convex regions are about $[2\xi a]^{1/2} \sim$ 11 Å). Such a large deformation is the cause of generating a force as large as 6 $k_BT$ per angstrom (Fig. 3 and Eq. IV.15), or 2.5 × $10^{-5}$ dynes. In comparison, if the surface tension were pulling in the direction of the channel axis, the force would be at most 2.5 × $10^{-8}$ dynes/Å times $2\pi r_0 \approx$ 1.6 × $10^{-6}$ dynes.

In conclusion, the gramicidin example demonstrates the importance of using the complete free energy for mem-

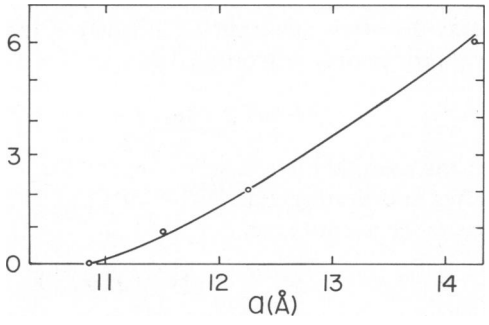


FIGURE 3 The effect of bilayer thickness on gramicidin channel lifetime. The ordinate is $ln\,\tau^{-1}$ with $\tau$ being the channel lifetime. The abscissa is the half-bilayer thickness a. The small circles are the measurement by Elliott et al. (1983). The curve is the theoretical expression Eq. IV.21 with $s = -3.9$, $t = -4.3$, $z$ = 1 Å. Both are normalized to zero at $a$ = (21.7/2) Å.

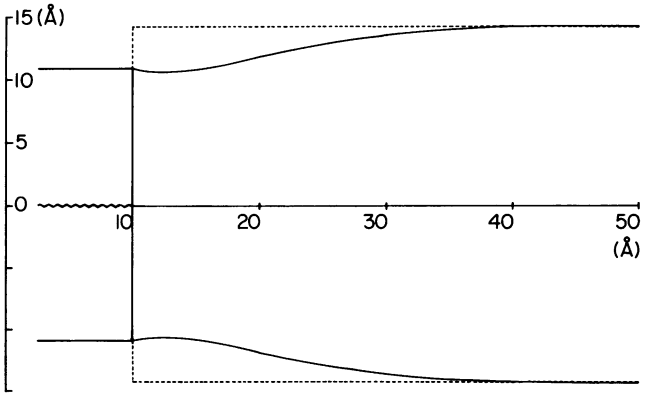


FIGURE 4 The shape of deformation—the cross section of a bilayer with a gramicidin channel embedded in it. The unperturbed (*dotted lines*) thickness of the hydrocarbon core of the bilayer is 28.5 Å. The length of the lipophilic exterior of the channel is 21.7 Å. The outside radius of the channel is 10 Å.

brane deformation. In particular, the inclusion of the splay term in the free energy may drastically change the shape and range of membrane deformations as well as the strength of protein-lipid interactions.

## APPENDIX

### Ensemble Averages

The emsemble average of a function of $D$, say $X(D)$, is defined as

$$\langle X(D)\rangle = \int \prod_{\mathbf{k}>0} dD_{\mathbf{k}} dD_{-\mathbf{k}}\, X(D) e^{-\mathcal{F}_D/k_BT} \Big/ \cdot \int \prod_{\mathbf{k}>0} dD_{\mathbf{k}} dD_{-\mathbf{k}} e^{-\mathcal{F}_D/k_BT} \quad \text{(A1)}$$

Since displacement $D(\mathbf{r})$ is a real quantity, its Fourier amplitudes $D_{\mathbf{k}}$'s are complex. Furthermore we have $D_{\mathbf{k}} = D^*_{-\mathbf{k}}$ according to Eq. II.8. The integration in Eq. A1 is understood as

$$\int dD_{\mathbf{k}} dD_{-\mathbf{k}} = \int d(ReD_{\mathbf{k}})\, d(ImD_{\mathbf{k}}), \quad \text{(A2)}$$

and the limits of integration for $ReD_{\mathbf{k}}$ or $ImD_{\mathbf{k}}$ are from $-\infty$ to $+\infty$. Because $\mathcal{F}_D$ is quadratic in $D_{\mathbf{k}}$, $\langle D^n\rangle = 0$ for any odd integer $n$. Straightforward calculations give the following results

$$\langle D^2\rangle = \sum_{\mathbf{k}} \langle D_{\mathbf{k}} D_{-\mathbf{k}}\rangle, \quad \text{(A3)}$$

$$\langle D_{\mathbf{k}} D_{-\mathbf{k}}\rangle = \frac{2k_BT/L^2}{aK_1k^4 + \gamma k^2 + \bar{B}/a}. \quad \text{(A4)}$$

The sum $\Sigma_{\mathbf{k}}$ can be replaced by an integral

$$\sum_{\mathbf{k}} = \frac{L^2}{(2\pi)^2} \iint dk_x dk_y = \frac{L^2}{2\pi} \int_0^{(2\pi/\lambda_0)} k dk. \quad \text{(A5)}$$

Eq. III.4 is the result of such an integration. The key steps in the calculation of $\langle D^4\rangle$ are

$$\langle D^4\rangle = 3\left\{4 \sum_{\mathbf{k}_1>0} \sum_{\substack{\mathbf{k}_2>0 \\ \mathbf{k}_2\neq\mathbf{k}_1}} \langle D_{\mathbf{k}_1} D_{-\mathbf{k}_1} D_{\mathbf{k}_2} D_{-\mathbf{k}_2}\rangle + \sum_{\mathbf{k}} \langle (D_{\mathbf{k}} D_{-\mathbf{k}})^2\rangle\right\}, \quad \text{(A6)}$$

$$\langle D_{\mathbf{k}_1} D_{-\mathbf{k}_1} D_{\mathbf{k}_2} D_{-\mathbf{k}_2}\rangle = \langle D_{\mathbf{k}_1} D_{-\mathbf{k}_1}\rangle \langle D_{\mathbf{k}_2} D_{-\mathbf{k}_2}\rangle \quad \text{for } \mathbf{k}_2 \neq \pm \mathbf{k}_1, \quad \text{(A7)}$$

$$\langle (D_{\mathbf{k}} D_{-\mathbf{k}})^2\rangle = 2\langle D_{\mathbf{k}} D_{-\mathbf{k}}\rangle^2. \quad \text{(A8)}$$

The combination of Eqs. A6, A7, and A8 gives

$$\langle D^4\rangle = 3\langle D^2\rangle^2. \quad \text{(A9)}$$

Consequently, we have Eq. III.3.

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