Calculation of deformation energies and conformations in lipid membranes containing gramicidin channels

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ABSTRACT In this paper we calculate surface conformation and deformation free energy associated with the incorporation of gramicidin channels into phospholipid bilayer membranes. Two types of membranes are considered. One is a relatively thin solvent-free membrane. The other is a thicker solvent-containing membrane. We follow the approach used for the thin membrane case by Huang (1986) in that we use smectic liquid crystal theory to evaluate the free energy associated with distorting the membrane to other than a flat configuration. Our approach is different from Huang, however, in two ways. One is that we include a term for surface tension, which Huang did not. The second is that one of our four boundary conditions for solving the fourth-order differential equation describing the free energy of the surface is different from Huang's. The details of the difference are described in the text. Our results confirm that for thin membranes Huang's neglect of surface tension is appropriate. However, the precise geometrical form that we calculate for the surface of the thin membrane in the region of the gramicidin channel is somewhat different from his. For thicker membranes that have to deform to a greater extent to accommodate the channel, we find that the contribution of surface tension to the total energy in the deformed surface is significant. Computed results for the shape of the deformed surface, the total energy in the deformed surface, and the contributions of different components to the total energy, are presented for the two types of membranes considered. These results may be significant for understanding the mechanisms of dimer formation and breakup, and the access resistance for ions entering gramicidin channels.

INTRODUCTION

The mechanics of lipid bilayer membranes containing gramicidin channels are of interest for two reasons. Firstly, the membrane mechanics are probably of great importance in understanding the initial inclusion of the gramicidin molecule into the membrane and, once the molecule is included, the kinetics of channel formation and breakup. A very clearly written review of the data and some of the important theoretical issues pertaining to the effects of the lipid membrane on gramicidin channel formation and breakup is given in Hladky and Haydon (1984). Since then, the theory of this problem has been significantly advanced by the explicit introduction of liquid crystal theory by Huang (1986). Relevant experimental data is to be found in the papers of Bamberg and Läuger (1973), Zingsheim and Neher (1974), Veatch et al. (1975), Bamberg and Benz (1976), Apell et al. (1977), Kolb and Bamberg (1977), Hendry et al. (1978), Rudnev et al. (1981), and Elliott et al. (1983). A second reason for exploring the membrane mechanics in the vicinity of gramicidin channels is that there is substantial theoretical and experimental evidence that a significant component of resistance to transmembrane flux in the gramicidin-lipid system is in resistance between the major portion of the bulk solution and the mouth of the channel (Läuger, 1976; Anderson, 1983*a*, *b*, and *c*; Levitt, 1985; Dani, 1986; Hainsworth and Hladky, 1987; Decker and Levitt, 1988; Levitt and Decker, 1988; Chiu and Jakobsson, 1989). The geometry of the lipid surface near the channel must be known to calculate this access resistance accurately (Jordan, 1987). There is no way at present to observe this geometry directly; it must be inferred from a theory of membrane mechanics.

A convenient and appropriate way to describe a bilayer lipid membrane is as a two-layer smectic-A liquid crystal. A smectic-A type liquid crystal is organized such that the constituent molecules are arranged roughly parallel to each other, with their principle axes perpendicular to the surface layer. A bilayer membrane has the polar head groups forming the equidistant parallel surfaces with the lipids in the interior oriented approximately perpendicular to those surfaces. There is a well-developed body of theory describing the elastic properties of such crystals (de Gennes, 1974; Stephen and Straley, 1974) which has been applied to the gramicidin-lipid system (Huang, 1986) for the special case of relatively thin membranes (low dielectric region <30 Å thick). The theoretical significance of the thin membrane assumption is that it permits neglect of the surface tension term in the expression for the free energy of a membrane deformed by the inclusion of a gramicidin channel.

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The present paper extends the work of Huang (1986) by including the surface tension term and by introducing the boundary condition that the gradient of the lipid thickness at the channel-lipid boundary adjusts itself to minimize the free energy of the lipid deformation induced by the channel insertion. These extensions permit us to test the assumptions Huang used in solving the thin membrane case and to calculate by numerical means the energetics of membranes of arbitrary thickness. The results of these numerical calculations are the main results of this paper.

EQUATIONS AND METHOD OF SOLUTION FOR THE LIPID SURFACE NEAR THE GRAMICIDIN CHANNEL

Huang (1986) has presented the correct equations for describing the lipid surface near the gramicidin channel, when the channel is embedded in a solvent-free membrane. In this section we briefly recapitulate those equations and then state our choice of boundary conditions and methods of solution, pointing out the ways in which our computations are similar to Huang's and the ways in which they are different. Then we present the modifications necessary to deal with a solvent-containing membrane.

In our calculations as in Huang's, cylindrical symmetry about the center of the channel lumen is assumed. Fig. 1 *a* shows a model channel-membrane system. The gramicidin channel's axis is coincident with the Z-axis, a is the half-bilayer thickness, r_0 is the distance from the channel centerline to the channel-membrane contact point, and u represents the position-dependent displacement from unperturbed half-thickness of the membrane. The free energy change unit per area is

$$F = F_0 + a\bar{B}(u/a)^2 + aK_1(\partial^2 u/\partial x^2 + \partial^2 u/\partial y^2)^2 + \gamma[(\partial u/\partial x)^2 + (\partial u/\partial y)^2]. \quad (1)$$

F_0 is the unperturbed membrane free energy; $\bar{B}$, K_1, and γ are the elastic deformation coefficients of compression, splay, and surface tension, respectively. To determine the minimum energy conformation, minimize the free energy with respect to variations in $u(x, y)$ and get the linear differential equation

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

where

$$\Delta = \frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2}. \quad (3)$$

We seek a numerical solution to Eq. 2 subject to the appropriate boundary conditions. The condition of radial

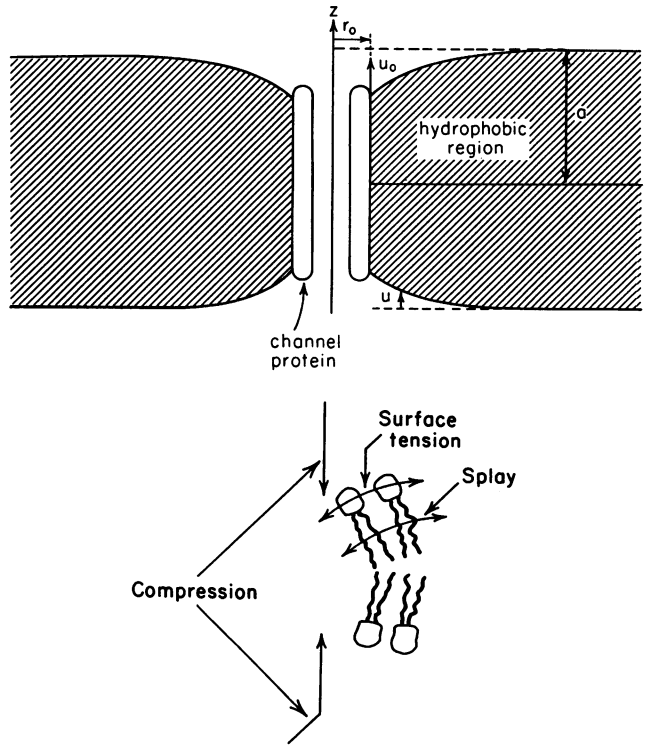

FIGURE 1 (*a*) Schematic cross-section (not to scale) of a gramicidin channel inserted in a phospholipid membrane. Because lipophilic exterior of the channel polypeptide does not generally have the same length as the width of the channel hydrophobic region, the membrane must be distorted in roughly the manner shown for the channel to be inserted. (*b*) Components of membrane distortion that contribute to the free energy and are represented by terms in Eq. 1. Surface tension involves changes in the density of the polar head groups along the surface of the membrane. Splay involves deviation from parallel of the average orientation of the phospholipid hydrocarbon chains. Compression involves changes in the membrane thickness.

cylindrical symmetry leads to

$$\Delta = \frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial}{\partial r}\right) \quad (4)$$

and the differential equation is

$$K_1(u'/r^3 - u''/r^2 + 2u'''/r + u^{IV}) - (\gamma/a)(u'/r + u'') + (\bar{B}/a^a)u = 0. \quad (5)$$

On a continuum basis Eq. 5 describes the elastic bending of a lipid bilayer. A membrane system is described completely here in terms of the macroscopic coefficients and by the assignment of the proper boundary conditions. Finally, Eq. 1 can be transformed similarly to cylindrical polar coordinates and put in integral form to yield

$$\mathcal{F} = 2\pi \int r dr \left[\frac{\bar{B}}{a}u^2 + aK_1\left(\frac{u'}{r} + u''\right)^2 + \gamma(u')^2\right]. \quad (6)$$

$\mathcal{F}$ is the free energy excess, relative to a planar equilibrium state, for a membrane deformation.

Eq. 5 is a fourth-order differential equation in r and thus requires four boundary conditions for a solution. They are, in general form:

$$u(r = r_0) = u_0 \quad u(r \rightarrow \infty) = 0$$
$$\frac{\partial}{\partial_r} u(r = r_0) = S \quad \frac{\partial}{\partial_r} u(r \rightarrow \infty) = 0. \tag{7}$$

Above, u_0 is the linear distance, in the Z-direction, between the lipophilic termination point of the gramicidin channel and the unperturbed average hydrophobic width of the lipid bilayer. In accordance with the results of Elliott et al. (1983) we assign the gramicidin lipophilic exterior length a value of 21.7 Å, as does Huang (1986). (This is the bilayer width of which further reduction does not effect meaningfully the mean channel lifetime for gramicidin in monoacylglycerol-squalene bilayers.) We also follow Huang in assigning a value of 10 Å for r_0. The hydrophobic thickness is determined experimentally via electrical capacitance or optical reflectance measurements (Hanai et al., 1964; Cherry and Chapman, 1969; respectively), and will vary from one type of membrane system to another. The two boundary conditions for $r \rightarrow \infty$ state that one requires the lipid bilayer surface to return to an undistorted conformation at some arbitrarily long distance from the gramicidin inclusion. s is the first derivative at the gramicidin-bilayer interface. In terms of the model system s represents the gramicidin lipophilic region-hydrophobic lipid membrane contact curvature. We have no a priori knowledge of the correct value of s. Therefore we go through a trial and error process of assuming a value of s, calculating a full surface and its free energy, assuming a new value of s, and so on, until we find the value of s that minimizes the free energy. This approach is conceptually similar to an earlier calculation of phospholipid vesicle shapes by global minimization of free energy in the surface lipid structure (Deuling and Helfrich, 1976). The details of our calculations are given below.

Implicit in the assignment of the two boundary conditions (Eq. 7) at the polypeptide-lipid interface is the assumption that the conducting polypeptide has little or no radial elasticity; i.e., it is essentially of fixed radius. Supporting evidence is that the circular dichroism of the "channel" form (head-to-head single helix) that is predominant in the lipid bilayer is unaffected by the presence of ions in the channel lumen (Wallace, 1986). If such a large force as that exerted electrostatically by an ion does not change the helical pitch (and hence the radius) of the channel, we judge that the channel structure will not be radially deformed by the interaction with the lipid. We note that this argument would not hold for the "pore" form (double helix) because the circular dichroism studies on this form show evidence for substantial change in pore radius with ion occupancy (Wallace, 1986).

All calculations were done on a Micro Vax II (Digital Equipment Corporation; Marlborough, MA). Eq. 5 was solved numerically using a variable order, variable step size, finite-difference method with deferred corrections (Pereyra, 1978). The numerical routine was provided as a package called DVCPR, by IMSL, Inc. (Houston, TX). This package required no modification for solving the surface of a solvent-free membrane for which it is reasonable to postulate that the compressibility coefficient is constant. Solving the set of Eqs. 5 and 6 involved a trial and error, or inductive, method. As a first step the two $r \rightarrow \infty$ boundary conditions were assumed to pertain at a particular large distance from the channel. (For example $u \rightarrow 0$ at $r = 80$ Å for the monoacylglycerol-squalene membrane.) u_0 in Eq. 7, as previously explained, is accurately predetermined. Then Eq. 5 was solved as a function of the variable parameter s (Eq. 7) with a linear mesh step size of 0.1 Å and the data was stored as input to the free energy integral Eq. 6. The computer solution of Eq. 5 returns the values of u, u', u'', and u''' at every grid point r. Finally, Eq. 6 was integrated separately at every value of s and the proper conformation was picked as the one which had the lowest free energy. The mode of integration was by the trapezoidal rule method with finite step sizes of 0.1 Å (Burden and Faires, 1985). Once s was determined, the $r \rightarrow \infty$ boundary conditions were further refined by successively reducing the radial deformation length and repeating the calculational procedure outlined above. The proper radial deformation length was then fixed at the minimum length whereby further reduction would produce a noticeable effect on the free energy. (In practice the curve described by the solution of Eq. 5 is well behaved and asymptotically goes to zero in a smooth way.) Similarly, the calculational process was repeated until s, the slope of the hydrophobic membrane surface at the gramicidin-membrane interface, was found to three significant figures.

The above equations differ from those solved by Huang (1986) in two ways. One is that in his analytical calculations Huang neglected the surface tension term in Eq. 1. Our numerical solutions of the full equations will confirm that this neglect is justified for the thin solvent-free membrane that Huang treated by analytical theory. The second difference is in the assignment of the fourth boundary condition (Eq. 7), for the contact angle between the lipid hydrophobic surface and the channel polypeptide. Huang's treatment in effect adjusted this angle so that the energy difference in the lipid surface associated with separating the monomers by 1.0 Å would correspond to the changes in channel lifetime with membrane thickness interpreted by transition state (Eyring) theory with a

constant preexponential coefficient. Our numerical solutions with the contact angle adjusted to give a surface of minimum free energy will be seen to differ from Huang's.

The above set of equations and boundary conditions are suitable for calculating the surface shape and the deformation energy of thin, relatively solvent-free glycerol-monooleate bilayer that was dealt with by Huang (1986). The appropriate values for the physical membrane parameters that enter into the equations are given below and also tabulated in Table 1. An appropriate value of the splay constant, K_1, is 10^{-6} dynes (Helfrich, 1973; Schneider et al., 1984; Engelhardt et al., 1985). The tension coefficient γ is 1.5×10^{-8} dyn/Å^2, (Hladky and Gruen, 1982) and the compression coefficient $\bar{B}$ is 5.0×10^{-8} dyn/Å^2 (White, 1978; Hladky and Gruen, 1982).

The other type of membrane we considered, because it is commonly used in experiments, is phosphatidylcholine solvate in *n*-decane. Also, phosphatidylcholine is an abundant component of biological membranes. For this membrane of the hydrophobic width ($2a$) is 48.0 Å (Fettiplace et al., 1971). Because the splay coefficient is determined by the crowding of the lipid chains when the surface is deformed, the value of this coefficient should be about the same for this type of membrane as for the solvent-free glyceryl monooleate membrane. For surface tension, we will set γ to 8.0×10^{-9} dyn/Å as determined by Neher and Eibl (1977), where a 1-M KCl bath solution was used. There is another value in the literature for this system: 2.41×10^{-8} dyn/Å (Requena and Haydon, 1975) for a 0.1-M NaCl electrolyte solution. It seems possible that the surface tension is a systematic function of electrolyte species and strength.

For the solvent-containing membrane, the compressibility is a more complicated function of the deformation than for the solvent-free membrane case. Two different types of processes seem likely to be involved in the compression of the solvent-containing membrane in the vicinity of the gramicidin channel. These are illustrated in the cross-section of the membrane shown in Fig. 5. We assume that far from the channel, forces causing compression would be acting on two resistances in series: one for compressing the hydrocarbon chains attached to the phospholipids and the other for squeezing solvent out from between the phospholipid layers. The compressibility coefficient of two media in series is given by:

$$\bar{B} = \bar{B}_1\bar{B}_2/(\bar{B}_1 + \bar{B}_2). \quad (8)$$

It can be seen from the above equation that if two media with quite different compressibilities are in such a "sandwich" arrangement, the compressibility of the "sandwich" will be very close to that of the most easily compressible, or softer, medium. A macroscopic analogy to compression of the solvent-containing membrane might be egg salad between two slices of firm pumpernickel bread. If one starts to compress this sandwich, to a reasonable approximation the compressibility will be that for squeezing egg salad out from between the bread until the slices of bread come into contact, at which time the compressibility will become that of the bread itself. Similarly, we assume that far from the channel in the solvent-containing membrane, compression would be by squeezing solvent out from between the phospholipid bilayers. Closer to the channel, the compression would be by pressing phospholipid chains together. The compressibility coefficient is distinctly different for the two regions. For the region farther from the channel where the membrane is thicker, we set the compressibility coefficient $\bar{B}$ at 5.75×10^{-11} dyn/Å^2. Hladky and Gruen (1982) obtained this value for $\bar{B}$ (as $\bar{B}/[2a]$) for glyceryl monooleate in *n*-decane by a procedure outlined in their Appendix III. (The compressibility properties of phosphatidylcholine/*n*-decane and glyceryl monooleate/*n*-decane membranes are nearly identical as determined by measurements of capacitive thickness change with applied transverse electric fields [Requena and Haydon, 1975].) For the region closer to the gramicidin inclusion the compressibility coefficient is calculated according to the method of Hladky and Gruen (see above), where the voltage dependent compressibility is determined experimentally (Alvarez and Latorre, 1978) for an unsolvated phosphatidylethanolamine bilayer system. In this case $\bar{B}$ is set to 5.36×10^{-8} dyn/Å^2. The dividing line between the two regions is set at u = 9.5 Å. This is the deformation required to bring the membrane hydrophobic surface to the nonsolvated phosphatidylcholine membrane equilibrium width, which is 29.0 Å (Alvarez and Latorre, 1978).

For a deformation greater than u = 9.5 Å, compression of opposing lipid chains occur. Now, the calculation of the

TABLE 1 Parameters used to calculate the free energy of the distortion in the phospholipid membrane around the incorporated gramicidin channel.

Type of membrane	Unperturbed thickness	Compressibility	Splay	Surface tension
	Å	*dyn*/Å^2 × 10^8	*dyn* × 10^6	*dyn*/Å × 10^9
Glycerylmonooleate (Solvent free)	28.5	5.0	1.0	15
Phosphatidylcholine (contains solvent)	48.0	5.36 (interacting hydrocarbon chains) 0.00576 (solvent movement)	1.0	8.0

Relevant equations and literature citations are given in the text.

deformation free energy is a bit modified. Eq. 6 still holds for the region farther from the channel, where the solvent is being pushed out from between the lipid chains, and $\bar{B}$ is the compressibility coefficient for the solvent-containing membrane. But close to the membrane where the lipid chains are being pushed together, the deformation energy is given by:

$$\mathcal{F} = 2\pi \int r\,dr \left[\frac{\bar{B}_{so} u_c^2}{a} + \frac{\bar{B}_{st}(u - u_c)^2}{a - u_c} + aK_1\left(\frac{u'}{r} + u''\right)^2 + \gamma(u')^2 \right]. \quad (9)$$

$\bar{B}_{so}$ is the "soft" compressibility coefficient, for the solvent to get pushed out of the way, $\bar{B}_{st}$ is the "stiff" compressibility coefficient for the lipid chains to get pushed together, and u_c is the "critical" value of u at which the compressibility coefficient changes value. The IMSL routine for solving the differential equations was modified appropriately to account for the change in the compressibility coefficient in different regions of the membrane.

RESULTS

The first calculations we present are for the solvent-free glycerolmonooleate bilayer, with an unperturbed hydrophobic width of 28.5 Å. For this membrane, Fig. 2 *a* shows the total free energy for each component (splay, surface tension, and compression) as a function of the gradient at the gramicidin-bilayer contact point. We can see from Fig. 2 *a* that the dominant energy terms resisting deformation are compression and splay. The free energy of surface tension for this bilayer system is negligible by comparison, nowhere comprising more than 8% of the total energy. The free energy surface is minimized when the radial gradient of u at the interface is -0.45.

Fig. 2 *b* shows the total integrated free energy of Eq. 6, also as a function of the gradient at the gramicidin-bilayer boundary. Of interest is the unambiguous potential energy minimum at the optimum gradient. As can be seen from the vertical energy scale, excursions from the minimum energy conformation can be very costly in free energy terms. The minimum energy conformation has a free energy of deformation of 3.91 k_bT. Referring to Fig. 2 *a* one can see that the splay energy function is a relatively strong function of the contact gradient, in contrast to the compression energy term which has a much weaker dependence on contact gradient. Thus, the minimum energy conformation is one which approximately minimizes the splay energy contribution.

Fig. 3 shows a cross-sectional view of the shape of the minimum energy surface. This figure shows the position of the hydrocarbon chain-head group boundary as a function of the radial distance from the channel centerline. The radial distance from the channel over which deformation persists is ~18 Å, or 2–3 monoacylglyceryl head group molecules (Fettiplace et al., 1971). Thus, representing the surface by a continuous curve is a fairly extreme idealization. Also, Fig. 3 depicts the lowest

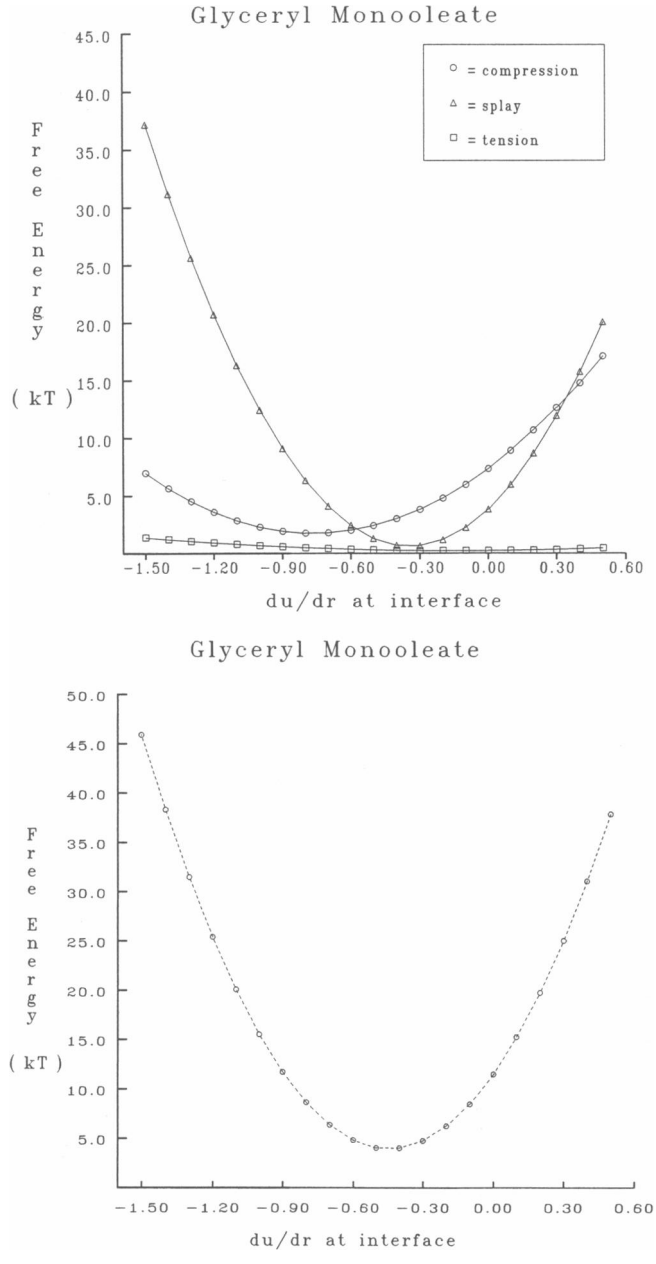

FIGURE 2 Results of calculations of deformation free energy for the thin glycerolmonooleate membrane. (*a*) shows free energy of each component as a function of assumed gradient of membrane thickness at the protein-lipid interface. Symbols are: ○ for compression, △ for splay, and □ for surface tension. (*b*) shows total free energy as a function of the assumed gradient.

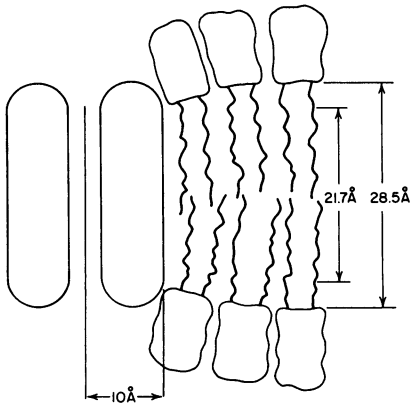

FIGURE 3 Cross-section of the minimum energy conformation for a gramicidin channel inserted into the glycerylmonooleate membrane, with the contact slope set at the minimum energy value indicated in Fig. 2.

energy equilibrium conformation which is a static representation. The actual structure is subject to thermal fluctuations. However, the strong dependence of the free energy on the the value of s (Figs. 2 *b* and 4 *b*) argues that most of the fluctuations are rather small, and that most of the time the shape is similar to the minimum energy conformation.

Figs. 4 and 5 are analogous to Figs. 2 and 3 (above), applied to a solvent-containing phosphatidylcholine in *n*-decane membrane with an unperturbed thickness of 48 Å. From Fig. 4 *a* we can see the relative importance of the separate energy components. For this membrane, surface tension is a more significant factor than for the solvent-free membrane, but compression energy is still the biggest single component in the minimum free-energy conformation, which has a gradient of -0.68 at the gramicidin-bilayer contact point (Fig. 4 *b*). This is a significantly steeper gradient than for the glycerylmonooleate membrane. A major cause of the difference seems to be the different form of the splay energy curve as a function of the contact gradient. Near the most energetically favorable contact gradient, there is an almost equal trade-off between increasing surface tension energy and decreasing compression energy as the contact gradient is increased. As a result, the minimum energy structure is one where the splay distortion term is approximately minimized, a result similar to that for the glycerylmonooleate membrane.

Fig. 4 *b* shows the total integrated free energy for the phosphatidylcholine in *n*-decane membrane. The minimized equilibrium conformation has a deformation energy of 3.09 k_bT. Interestingly, this is a bit less than that for the thinner membrane calculated above. When the higher experimental value for the surface tension coefficient was assumed, as discussed in Equations and Method above, essentially the same shape of the energy curves and location of minima was found as in Fig. 4, but total free energy at the minimum energy conformation was 5 k_bT. Fig. 5 is a cross-sectional view of the radial deformation region for the phosphatidylcholine bilayer. As can be seen, the channel-produced deformation is approximately completely contained within a radius of 100 Å, at which point the vertical bilayer displacement is $0.05u_0$. The major radial deformation therefore encompasses 11–13 phospholipid molecules (Fettiplace et al., 1971; Hauser et al., 1981). Of note also is the radial position where solvent is just excluded. This corresponds to a point where the hydrophobic membrane surface is deformed to u = 9.5 Å which has a radial coordinate of 17.1 Å. This is sufficient to accommodate one phosphatidylcholine molecule before solvent intrudes.

The surface tension term in Eq. 1 is a linear approximation to a nonlinear expression given in radial coordinates as:

$$2\gamma\left\{\left[1+\left(\frac{\partial u}{\partial r}\right)^2\right]^{1/2}-1\right\}. \qquad (10)$$

To test the validity of the linear approximation we calculated the surface tension energy component for each assumed contact angle, by substituting the previously calculated spatial derivative, u', into expression 10 and integrating over the surface contour. Nowhere over the range of Fig. 4 *a* did the calculated difference between linear and nonlinear surface tension exceed 12%. At the minimum energy conformation the difference amounted to 3%. These differences are not enough to modify any of our results significantly. In the unsolvated glycerylmonooleate bilayer, where the deviation from the flat membrane is less extreme than for the solvent-containing membrane and the surface tension term is very small, the linear approximation is even better.

As the surface tension term in Eq. 1 is an approximation, so also is the splay term. The Laplacian operator in the splay term is an approximation to the total curvature of the membrane, which is defined as the divergence of the projection on the $u = 0$ plane of the gradient of u (Peliti and Leibler, 1985):

$$H = \text{div (grad } (u)/\{1 + [\text{grad } (u)]^2\}^{1/2}), \qquad (11)$$

where H is the curvature. By inspection of Eq. 11, one can see that when grad (u) is much less than 1, H can be approximated by div [grad (u)]. This is the form of the splay curvature given in Eq. 1. The validity of the splay approximation was tested in an analogous fashion to that of the surface tension term as described above. The energy of the full curvature term was calculated by substituting the previously calculated gradient of u into

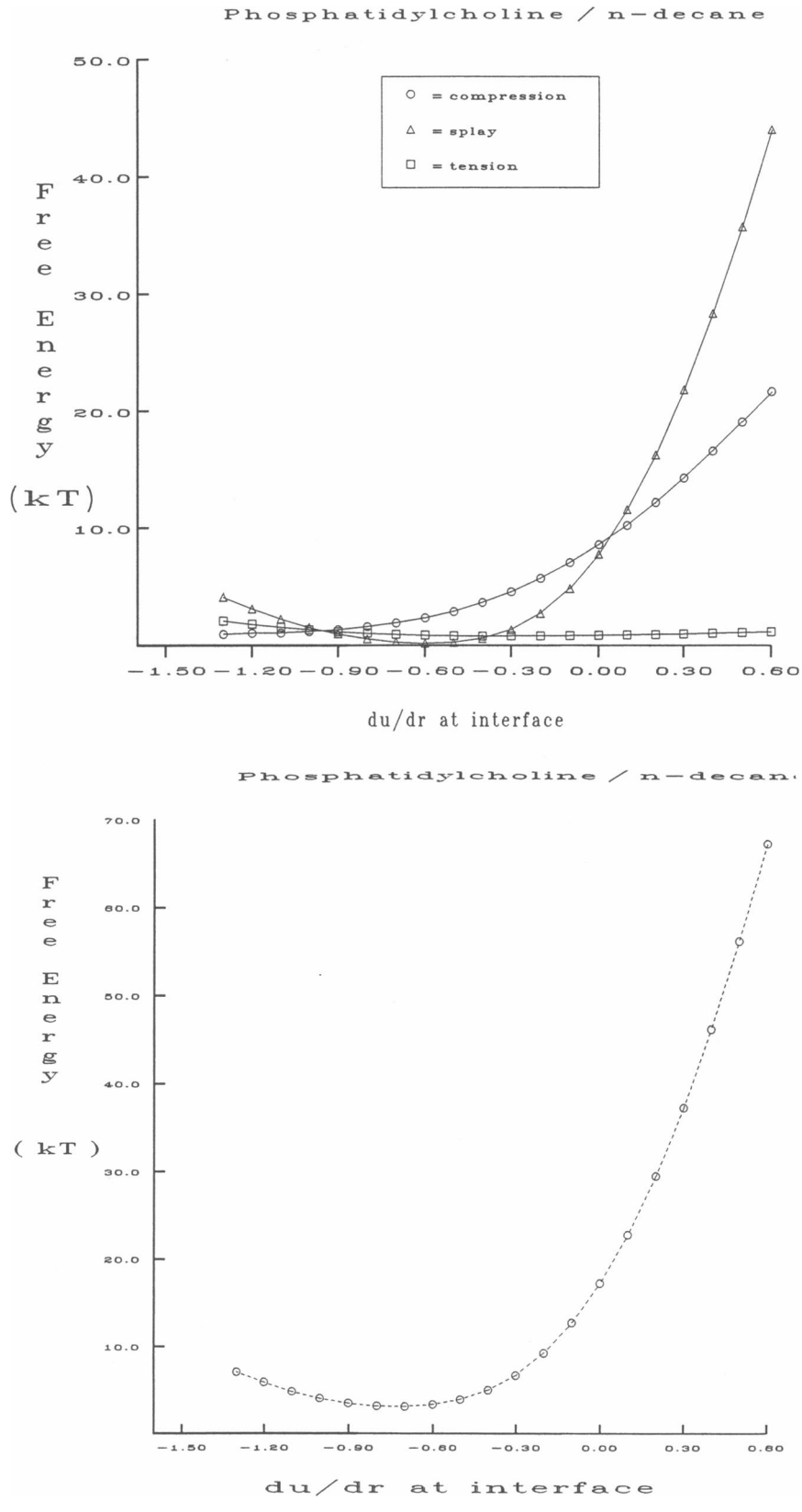

FIGURE 4 Same as Fig. 2, for relatively thick solvent-containing phosphatidylcholine membrane.

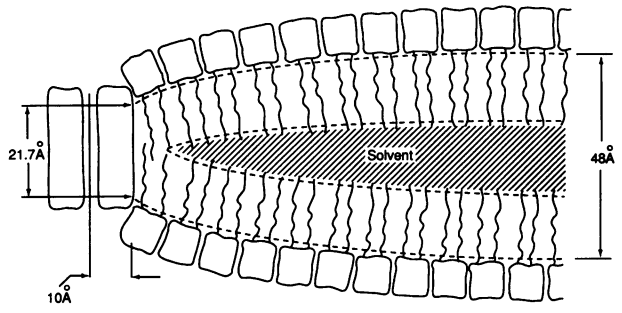

FIGURE 5 Same as Fig. 3, for relatively thick solvent-containing phosphatidylcholine membrane.

Eq. 11 for each of the previously solved contours. The difference between the total curvature energy and the splay was no more than a few tenths of kT except for contact angles much larger than those for the minimum energy contours. We therefore judge that our results are not significantly modified by this approximation.

SUMMARY and DISCUSSION

In this paper we have presented numerical solutions to the equations for free energy of deformation of lipid membrane in the vicinity of a gramicidin channel. We solved for the minimum free energy configuration for two cases of interest: a relatively thin and incompressible solvent-free membrane (glycerolmonooleate prepared from squalene) and a relatively thick solvent-containing membrane (phosphatidylcholine prepared from *n*-decane), which has a substantial region that is relatively easy to compress. The qualitative shapes of the minimum free energy surfaces for the two membranes are very similar. In both cases it turns out that the shape is assumed that approximately minimizes the splay component of the free energy, which is the most shape-dependent component. However, the gradient of the membrane thickness at the channel-lipid boundary is greater for the thick membrane, so the lipid dimpling around the edge of the channel forms a somewhat more confined "vestibule" for ion approach to the channel mouth in the case of the thick membrane compared with the thin membrane. The total free energy of membrane deformation is very similar for the two cases, being ~4 K_bT for the thin membrane and 3–5 k_bT for the thick membrane, depending on the value of surface tension assumed. In both cases the energy is small enough to suggest that thermal fluctuations in the membrane thickness should be of sufficient magnitude to bring monomers floating on opposite sides of the membrane into "docking" position for channel formation (see Fig. 6). This picture of monomers from opposite sides of the membrane joining to form a channel is consistent with the stoichiometry of channel formation from a charged (hence significantly water soluble) analogue of gramicidin A (Apell et al., 1977).

Although we use the same equations to describe the membrane free energy as does Huang (1986), our calculated shape for the thin membrane is different from his. This is because of differing choices for the boundary conditions. We elected a boundary condition that the radial gradient of membrane thickness at the channel-lipid boundary would adjust itself to minimize the total free energy of membrane deformation around the channel. Huang's corresponding boundary condition was that the channel lifetime kinetics as a function of membrane thickness could be described by an Arrhenius relationship with incremental activation energy being given by the differential membrane deformation energy as membrane thickness is varied. His calculation contains the further assumption that the conducting channel lifetime ends when the two monomers become separated by 1.0 Å. These assumptions are reasonable, but are also arbitrary. It is not known with any certainty how much the channel monomers must separate for the channel to become nonconducting, nor is there any independent evidence suggesting an Arrhenius relationship of the sort that was assumed. On the other hand the principle that a flexibile structure will relax toward a free energy minimum is of very general validity and thus, in our judgment, provides a

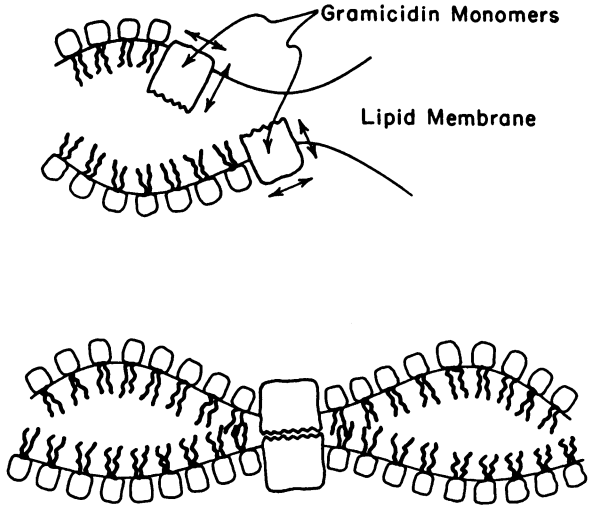

FIGURE 6 Diagram of possible mechanism for gramicidin channel formation suggested by calculations in this paper. Because membrane deformation energy per channel is of the order of thermal energy (Figs. 2 and 4), it is possible that gramicidin monomers floating in opposing membrane faces may be brought into docking position for channel formation by thermal fluctuations in membrane thickness.

surer basis for establishing boundary conditions for the liquid crystal equations.

In addition to shedding light on the energetics of channel association and dissociation, the conformation calculations for the lipid surface around the gramicidin channel will be useful in calculating the electric field around the mouth of the channel, essential for accurate calculations of channel access resistance.

E. Jakobsson had a useful conversation with Dr. H. Huang that was very helpful in our understanding of the application of the liquid crystal theory to biological membranes. The comments of the referees resulted in improvements in the manuscript. During the period we were revising the manuscript, additional conversations with Dr. Larry Scott and Dr. Paul Goldbart were useful.

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