Effect of Lipid Characteristics on the Structure of Transmembrane Proteins

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ABSTRACT The activity of embedded proteins is known to vary with lipid characteristics. Indeed, it has been shown that some cell-membrane proteins cannot function unless certain non-bilayer-forming lipids (i.e., nonzero spontaneous curvature) are present. In this paper we show that membranes exert a line tension on transmembrane proteins. The line tension, on the order of 1–100 kT/protein, varies with the lipid properties and the protein configuration. Thus, membranes composed of different lipids favor different protein conformations. Model predictions are in excellent agreement with the data of Keller et al. (*Biophys. J.* 1993, 65:23–27) regarding the conductance of alamethicin channels.

INTRODUCTION

Cell membranes are known to contain a large variety of lipids and proteins (Gennis, 1989). The need for many types of proteins is clear, because they are the biochemically active components. Presumably, the many lipid types must also play an important role in membrane function, because their presence and composition are closely regulated by the cell. Yet the role of specific lipids in cell functions has not been determined conclusively.

One may categorize lipids by their "spontaneous curvature," i.e., the curvature of a monolayer at a water-oil interface (Israelachvili, 1992). Bilayer-forming lipids have a spontaneous curvature of zero; non-bilayer ones may have either positive or negative values (Fig. 1). A significant fraction of cell lipids are non-bilayer-forming lipids. Previous studies established that such lipids facilitate fusion and transport by increasing membrane flexibility (Cullis and de Kruijff, 1979). However, this observation cannot fully account for the presence of many different non-bilayer-forming lipids in cell membranes.

Gruner (1985) suggested that because cells homeostatically adjust their curvature, some proteins must be sensitive to that curvature. More recent investigations suggest that there is indeed a correlation between the spontaneous curvature of the membrane and the performance of embedded proteins (Keller et al., 1993; Bezrukov et al., 1997; Lundbeak et al., 1997). Similarly, there have been reports that at least some cell proteins cannot function except in the presence of specific non-bilayer-forming lipids (Rietveld et al., 1995; Bogdanov et al., 1996). However, the mechanism by which lipid properties may affect protein performance is not understood. de Kruijff (1997) suggested that the configuration of embedded proteins is somehow related to a membrane-induced pressure which varies, in some manner, with lipid type. As a result, different lipids induce different protein configurations and differences in performance.

Theoretical studies of membrane-protein interactions tend to focus on membrane-induced interactions between proteins (Bruinsma and Pincus, 1996). Little attention has been paid to the effect of membrane characteristics on the configurations of embedded proteins. Recently, Cantor (1997a,b) has shown that membranes exert a transverse pressure profile on embedded proteins. However, the model used could not provide a direct correlation between the lipid characteristics and the preferred protein configuration.

In this paper we derive a simple relationship between lipid properties and the tension exerted on membrane proteins. We find that the tension exerted on a protein varies as a function of the lipid spontaneous curvature and the protein configuration. Thus, changes in the membrane composition may affect both the cross-sectional area of a protein and its configuration, as defined by the contact angle between the protein and the membrane (Fig. 2).

Bilayer-Protein Model

Bilayers are composed of two identical, tensionless monolayers. For simplicity, we discuss here only membranes composed of a single type of lipid or a homogeneous mixture. The area per lipid (Σ_0) and thickness (u_0) of the monolayers are determined by the lipid chemistry (Israelachvili, 1992). We define the dimensionless spontaneous curvature by $c_0 = u_0/R_0$, where R_0 is the radius of the optimal monolayer interface between oil and water (Fig. 1). To minimize tail-water contact, even non-bilayer forming lipids aggregate into bilayers in water despite inevitable packing frustrations (Ajdari and Leibler, 1991).

Embedded proteins perturb the packing of the lipids in the bilayer. The perturbation is defined by a normalized thickness $\Delta(x) \equiv u(x)/u_0 - 1$, where $u(x)$ is the thickness of the perturbed monolayer and x the distance from the inclusion boundary. We use ($'$) to denote a derivative with

Received for publication 24 February 1998 and in final form 13 June 1998.
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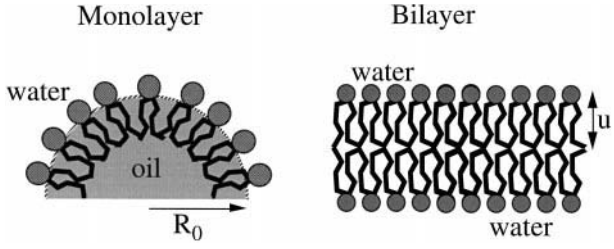

FIGURE 1 The spontaneous curvature of lipid monolayers is defined as the preferred interface curvature of the oil-water interface, a function of the head/tail size ratio. Bilayer-forming lipids are relatively symmetric and their oil-water interfaces are flat. Non-bilayer-forming lipids are not symmetric and the interface curves toward or away from the oil phase. In the bilayer, non-bilayer-forming lipids are frustrated. u_0 denotes the thickness of a flat unperturbed monolayer.

respect to x. The energy of a monolayer per unit circumference of the protein can be written (Dan et al., 1994; Dan and Safran, 1995) as

$$\gamma_m(\theta) = \int_0^\infty \frac{dx}{\Sigma_0}\{B\Delta^2 + u_0{}^4K(\Delta'')^2 + 2Kc_0u_0{}^2\Delta''(1+\Delta)\} \tag{1}$$

In the following discussion we will concentrate on membrane proteins whose thickness matches that of the bilayer. The boundary between the protein and the monolayer is therefore defined only by θ, the contact angle (Fig. 2). The first contribution in Eq. 1 is due to packing constraints; B is the monolayer compressibility, i.e., the energy penalty for perturbation of the local density from the equilibrium preferred value, Σ_0. The second contribution is due to the bending energy of the monolayer, where K is the bending modulus. The third term accounts for the energy cost when the interface curvature does not match the preferred spontaneous curvature. We do not address possible changes in the spontaneous curvature as a function of density. All energies are given in units of kT, where k is the Boltzmann coefficient and T the temperature.

Monolayers and bilayers are self-assembled. Thus they are free to adjust their structure in response to stimuli such as embedded proteins. We show the optimal thickness profile of a monolayer near a cylindrical protein ($\theta = 0$) in Fig. 3. The profile is calculated by minimization of the energy, γ_m (Dan and Safran, 1995). Despite the fact that there is no thickness mismatch between the hydrophobic regions of the protein and the monolayer, the thickness of the monolayer is not uniform. Rather, it displays a decaying oscillation as a function of distance from the protein. The oscillation amplitude is proportional to the lipid spontaneous curvature. Similar profiles are obtained for cases in which the contact angle, θ, is not zero, and for systems where there is a thickness mismatch between the protein and the monolayer (Dan et al., 1994; Dan and Safran, 1995; Aranda-Espinoza et al., 1996).

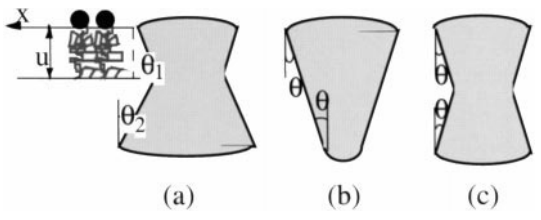

FIGURE 2 Protein-monolayer geometry. The protein thickness is equal to that of the unperturbed bilayer (u_0). x defines the distance from the protein boundary and u is the thickness of the monolayer at distance x. θ is the contact angle between the monolayer and the protein: (*a*) asymmetric, where $\theta_1 \neq \theta_2$; (*b*) conical, where $\theta_1 = -\theta_2 = \theta$; (*c*) symmetric (hourglass), where $\theta_1 = \theta_2 = \theta$.

RESULTS

The energy of a bilayer, γ_B, is given by the contributions of the two monolayers. Assuming that both contact angles are small yields (using the method described in Dan et al., 1994),

$$\gamma_B = \gamma_m(\theta_1) + \gamma_m(\theta_2) \approx \frac{\sqrt{2}u_0K}{a^{1/4}\Sigma_0}\{\theta_1{}^2 + \theta_2{}^2\} - \frac{2u_0Kc_0}{\Sigma_0}\{\theta_1 + \theta_2\} \tag{2}$$

where $a = K/B$, the dimensionless ratio between the bending and compression moduli. γ_B describes the line tension between the protein and the bilayer; it acts as a one-dimensional pressure on the protein circumference. When it is positive, the bilayer compresses the protein, whereas a negative value induces protein expansion.

The first contribution to the line tension is independent of the lipid spontaneous curvature and is always positive,

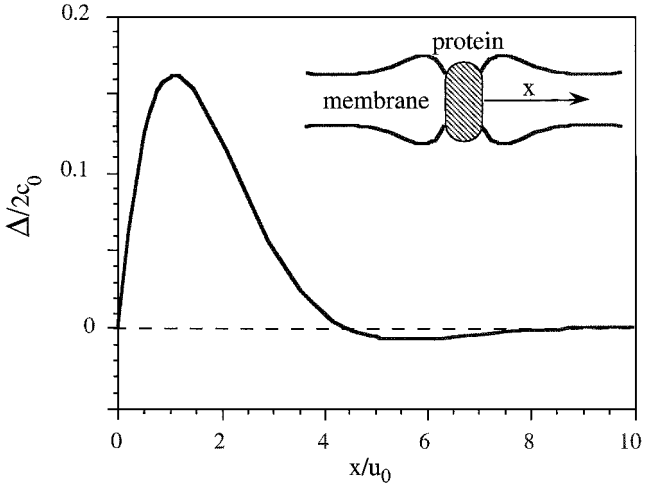

FIGURE 3 The perturbation profile of a membrane near a cylindrical protein of the same hydrophobic region thickness (*inset*). The distance from the protein edge, x, is normalized by the unperturbed monolayer thickness, u_0. The thickness profile of the monolayer is defined as $\Delta = u(x)/u_0 - 1$. Despite the thickness matching, there is a monolayer thickness perturbation near the protein. The perturbation amplitude is linearly proportional to c_0.

thereby promoting protein compression. The second contribution scales with c_0 and may be positive or negative. For cylindrical proteins where $\theta_1 = \theta_2 = 0$, the line tension is always zero, i.e., the bilayer is indifferent to the presence of such proteins, which would not feel any membrane-induced force to expand or contract.

If the protein molecular structure is rigid so that the contact angles are fixed, one may examine two limiting cases, as shown in Fig. 2. For conical proteins where $\theta_1 = -\theta_2 = \theta$,

$$\gamma_B \approx \frac{2\sqrt{2}u_0 K\,\theta^2}{a^{1/4}\Sigma_0} \tag{3a}$$

and for symmetrical (hourglass) proteins where $\theta_1 = \theta_2 = \theta$,

$$\gamma_B \approx \frac{2\sqrt{2}u_0 K\,\theta^2}{a^{1/4}\Sigma_0}\left\{1 - \frac{a^{1/4}\sqrt{2}\,c_0}{\theta}\right\} \tag{3b}$$

The line tension exerted on conical proteins is always positive and insensitive to the lipid spontaneous curvature. Any lipid packing gain (or loss) in one monolayer is offset by a loss (or gain) in the second monolayer.

In the case of symmetrical proteins there are two regimes. When the spontaneous curvature c_0 is lower than a critical value given by $(\theta/\sqrt{2}a^{1/4})$, the line tension is positive. However, if the spontaneous curvature is larger than this critical value the line tension becomes negative, thereby favoring protein expansion. This is due to the relief from packing constraints that the protein introduces into the monolayers. The highest line tension is exerted on proteins whose θ has the sign opposite that of the lipid spontaneous curvature, thereby tending to compress the protein even more than the bilayer-forming lipids. This is in agreement with the qualitative picture suggested by de Kruijff (1997).

If the protein is deformable (either through compression or by conformational changes), the line tension is minimized when both contact angles achieve an optimal value

$$\theta_1 = \theta_2 = \theta^* = \frac{a^{1/4}c_0}{\sqrt{2}} \tag{4a}$$

for which the line tension is

$$\gamma_B(\theta^*) = \frac{-\sqrt{2}u_0 K c_0^2 a^{1/4}}{\Sigma_0} \tag{4b}$$

As may be expected, bilayer-forming lipids, in which $c_0 = 0$, favor a cylindrical protein conformation. Non-bilayer-forming lipids favor a contact angle which is proportional to their spontaneous curvature. The line tension corresponding to this optimal angle is always negative, namely, promoting protein expansion.

DISCUSSION AND CONCLUSIONS

Testing these predictions requires data regarding the relationship between protein structure and membrane properties. Unfortunately, until recently the structure of proteins in membranes could not be measured. However, indirect methods which probe the function of proteins in membranes can be used to examine our model predictions.

Of special interest is the Keller et al. (1993) study of the relationship between membrane spontaneous curvature and the conductance of alamethicin channels. The membrane spontaneous curvature was controlled through changes in either composition (ratio of DOPC to DOPE, Keller et al., 1993) or pH (Bezrukov et al., 1995).

Alamethicin channels are characterized by different conductance levels. Keller et al. (1993) and Bezrukov et al. (1995) measured the conductance of a single channel embedded in membranes of various spontaneous curvatures. They found that the conductance levels were unaffected by the membrane properties, thereby indicating that the structure of the channel at each level is independent of the membrane spontaneous curvature. However, the fraction of time the channel stayed in each level was found to vary exponentially with the spontaneous curvature. Defining the probability that a single channel will be in level n relative to level 1 as R_n, the Keller et al. (1993) data show an exponential dependence of R_n on the membrane spontaneous curvature, as schematically sketched in Fig. 4.

Using Eq. (2), the energy of a channel conductance level n is proportional to $(\gamma_B{*}L_n)$, where L_n is the circumference of the channel at state n. We assume that the area for ion transfer at each level is independent of the membrane spontaneous curvature. This is in agreement with the observation of Keller et al. (1993) that the conductance at each level was, indeed, independent of the membrane's c_0, and we assume that the contact angles, θ_1 and θ_2 are the same regardless of the conductance level. We take this to mean that the line tension acting on the channel is the same at all

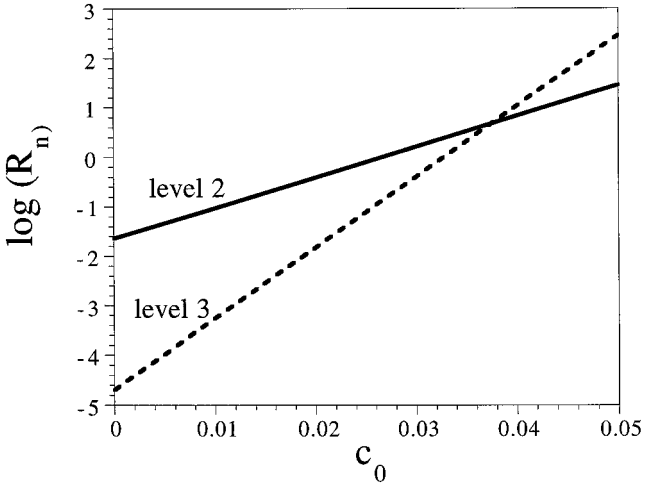

FIGURE 4 A schematic depiction of the Keller et al. (1993) data for the conductance levels of a single alamethicin channel. R_n denotes the probability of the channel being in the nth conductance level, relative to the probability of the first level. c_0 is the measured membrane spontaneous curvature. The data were fitted with the approximate linear relationships $\log(R_2) = -1.64 + 62\,c_0$ and $\log(R_3) = -4.6 + 143\,c_0$.

levels, and the only difference between them is the *area* available for ion transfer. The probability that a channel will be at the *n*th conductance level is given by a Boltzmann factor, $e^{-\gamma_B * L_n}$. The ratio between the times spent at each conductance level, R_n, is then

$$R_n \approx \frac{e^{-\gamma_B * L_n}}{e^{-\gamma_B * L_1}} = e^{-\alpha_n} * e^{\beta_n c_0} \tag{5}$$

where

$$\alpha_n = \frac{\sqrt{2} u_0 K (L_n - L_1)}{a^{1/4} \Sigma_0} \{\theta_1^2 + \theta_2^2\}$$

and

$$\beta_n = \frac{2 u_0 K (L_n - L_1)}{\Sigma_0} \{\theta_1 + \theta_2\}$$

Thus, we predict that R_n should scale exponentially with the spontaneous curvature. Also, because the area available for ion transfer increases with the channel level, $L_n > L_1$, and R so that $R_n \sim e^{+c_0}$. Examining the data (Keller et al., 1993) as sketched in Fig. 4, we see that R_n scales with c_0 in that manner. The ratio between the intercepts (at $c_0 = 0$) of level 2 and level 3 is approximately 0.35 (Keller et al., 1993), and should be proportional to $\alpha_2/\alpha_3 = (L_2 - L_1)/(L_3 - L_1)$. The ratio between the slopes should be the same, since it scales as $\beta_2/\beta_3 = (L_2 - L_1)/(L_3 - L_1)$. Indeed, the data suggest that the ratio between the slopes is approximately 0.43, which is within reasonable agreement with the intercept ratio. We conclude that our simple model is consistent the behavior of alamethicin channels embedded in bilayers of varying spontaneous curvature.

In conclusion, we show here that the conformations of embedded proteins may be controlled by the lipid type through a membrane-induced line tension. The magnitude of the line tension varies with the protein-membrane contact angle and the lipid spontaneous curvature, as well as the bilayer compression and bending moduli. Bilayer-forming lipids favor cylinder-shaped proteins. Non-bilayer-forming lipids favor symmetrical, or hourglass, protein conformations where both contact angles match the lipid spontaneous curvature. The functionality of a protein, which is related to its conformation in the membrane, may thus be controlled by the membrane lipids. The magnitude of γ_B is significant: typical numbers (Evans and Rawicz, 1990) yield a line tension of order $20\theta^2$ kT/Å, which is equivalent to 1–100 kT/protein. The direct correlation between lipid characteristics and the optimal configurations of embedded proteins, shown here, may help explain the need for a large variety of lipids in cell membranes; each protein requires a specific lipid to obtain its functional conformation. These predictions agree both qualitatively and quantitatively (within experimental error) with the experiments of Keller et al. (1993).

Although we examined only proteins whose thickness matches that of the bilayer, the model can be easily extended to cases where there is a thickness mismatch between the membrane and the protein (Aranda-Espinoza et al., 1996; Dan et al., 1994; Dan and Safran, 1995). Another effect which may be relevant is possible demixing due to a protein embedded in a bilayer composed of several lipid types (P. Sens and S. A. Safran, manuscript in preparation).

It should be noted that protein configurations may also be affected by a transverse membrane pressure profile, namely, the pressure as a function of distance from the hydrophobic-hydrophilic interface. Recently, Cantor (1997a,b) has calculated this effect and found it to be of the same order of magnitude as the line tension we present here. However, the pressure model does not provide an explicit relationship between spontaneous curvature and the pressure profile and thus cannot be used to estimate the effect of that parameter on protein shape. It is also unclear how sensitive this pressure profile is to the spontaneous curvature, especially if one accounts for area and thickness adjustments near the protein (which may be significant, as shown in Fig. 3).

We thank P. Nelson for bringing this problem to our attention and P. Pincus, R. Cantor, and especially P. Sens and S. Bezrukov for helpful discussions. SAS acknowledges the support of the Petroleum Research Fund administered by the American Chemical Society and the US-Israel Binational Science Foundation for their support.

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