ELSEVIER

Chemistry and Physics of Lipids
101 (1999) 45–56

CPL
CHEMISTRY AND PHYSICS OF LIPIDS

www.elsevier.com/locate/chemphyslip

The influence of membrane lateral pressures on simple geometric models of protein conformational equilibria

Robert S. Cantor *

Department of Chemistry, Dartmouth College, Hanover, NH 03755, USA

Abstract

The function of many intrinsic membrane proteins requires a conformational transition that is often strongly influenced by the molecular composition of the bilayer in which the protein is embedded. Recently, a mechanism for this shift in conformational equilibrium was suggested, in which it is argued that a shift in distribution of lateral pressures of the bilayer resulting from a change in lipid composition alters the amount of mechanical work of the protein conformational transition, if the change in the cross-sectional area profile of the protein varies with depth within the bilayer. As there is little information on the change in shape of the transmembrane region of any protein, various simple geometric models are considered. For both a generic model, and more specific models that approximate likely cooperative rearrangements of α-helices in bundles, it is found that the conformational equilibrium depends on the first and second integral moments of the lateral pressure distribution. In addition to revealing the possible physical underpinnings of the well-known correlation between protein activity and the 'nonlamellar' tendency of bilayer lipids, this dependence on moments of the pressure profile allows for prediction of the relative effects of different lipid compositional changes even in the absence of information on specific protein shape changes. Effects of variation in acyl chain length, degree and position of *cis*-unsaturation, and addition of cholesterol and small interfacially-active solutes (*n*-alkanols) are compared.

Keywords: Lateral pressure profile; Lipid bilayers; Lipid–protein interactions

1. Introduction

The function of an intrinsic membrane protein often requires a change in shape that implies a transition from an inactive to an active conformation. In general, the conformational equilibrium strongly favors the inactive state until an external stimulus causes a significant shift in the equilibrium toward the active state. The stimulus may be, for example, a change in electrical potential across the membrane or binding of ligands to sites on the protein, as is common for ion channel proteins, or the absorption of light, as for rhodopsin. In many cases, the activity of the protein, i.e. the distribution of its conformational states (both in the absence and the presence of the stimulus) is strongly affected by the molecular composition of the bilayer in which it is anchored (Mitchell et al., 1992; Bienvenüe and Sainte Marie, 1994; Litman

* Tel.: +1-603-646-2504; fax: +1-603-646-3946.
E-mail address: rcantor@dartmouth.edu (R.S. Cantor)

0009-3084/99/$ - see front matter
PII: S0009-3084(99)00054-7

and Mitchell, 1996; Mitchell et al., 1996; Brown, 1997; Rankin et al., 1997; Chu et al., 1998). Variation in lipid characteristics, such as head group type and the unsaturation and length of the acyl chains, or addition of cholesterol or small solutes such as alcohol and anesthetics can all contribute to modulation of protein function. The molecular mechanisms of such lipid–protein coupling remain poorly understood. While it is likely that direct interactions between some membrane molecular constituents and proteins play a significant role in many cases, such interactions are not considered in this work. Rather, the consequences of a nonspecific (indirect) mechanism are examined, i.e. in which a change in membrane composition alters a structural or thermodynamic property of the bilayer, which in turn shifts the conformational equilibrium of the protein embedded therein.

In recent work (Cantor, 1997a,b, 1998, 1999), a nonspecific mechanism has been proposed in which a change in bilayer composition causes a depth-dependent redistribution of lateral stresses (pressures) exerted by the bilayer, which alters the amount of mechanical work accompanying the conformational change of the protein, if in the bilayer region the protein laterally contracts or expands non-uniformly. For this hypothetical mechanism to be tested and to have predictive value, it is necessary to determine, either through calculations or measurements: (1) the lateral pressure profile as a function of bilayer lipid composition; and (2) the change in the shape of a given protein in the trans-membrane region. While it is possible to predict (if not to measure directly) the shifts in lateral pressure for different lipid composition, the second part is more elusive: there exists little direct experimental information on the change in the cross-sectional area (as a function of depth in the bilayer) that accompanies protein conformational transitions. Still, there is considerable evidence that for many superfamilies of intrinsic membrane proteins, the transmembrane domain is comprised of a bundle of α-helices, and that for such bundles, the protein may achieve its conformational change with a relatively small expenditure of free energy through a cooperative reorientation of the helices of the bundle, as has been reported for the nicotinic acetylcholine receptor (Unwin, 1993, 1995). In the present work, it is thus considered in what manner and to what degree various changes in the bilayer pressure profile would be predicted to influence the conformational equilibrium of a protein that undergoes various kinds of cooperative reorientations within the transmembrane domain. First, a simple geometic model is developed that provides a general description of a change in protein shape, i.e. the change in its cross-sectional area as a function of depth within the bilayer. Then, geometric models for two specific kinds of reorientations are developed, describing a cooperative tilt obtained by twisting a bundle of cylindrical helices, and a cooperative rotation in a collection of sharply bent rigid rods. In all cases, the resulting shifts in protein conformational equilibria are predicted to depend on the first and second integral moments of the pressure profile, P_1 and P_2, as defined in detail below. Thus, useful predictions can be made even in the absence of information on the change in shape of a given protein: bilayers of different composition, but which are predicted to have the same P_1 and P_2 should have similar effects on protein function, to the extent that coupling of the redistribution of lateral pressure with a change in the cross-sectional area profile is the dominant mechanism by which lipid composition affects protein function. The relative magnitudes of the effects of various changes in bilayer composition on protein activity can thus be predicted.

2. Lateral pressure profile in lipid bilayers

In a 'self-assembled' membrane, i.e. in the absence of any lateral constraints, the bilayer is free to adjust its molecular area (expand or contract laterally) so as to minimize its free energy. In other words, once equilibrium is reached, the sum of the forces acting in the plane of the bilayer (lateral pressures) is essentially zero. However, since the bilayer is of finite thickness, the various contributions to the total lateral pressure will in general act at different depths; positive lateral pressures occuring at some depth must therefore

be balanced by negative pressures (tensions) elsewhere (Israelachvili et al., 1980; Seddon, 1990; Ben-Shaul, 1995; Seddon and Templer, 1995; Cantor, 1997a, 1999). To be more explicit, imagine dividing up the bilayer into thin planar slices. Within a slice centered at a depth z in the bilayer, a nonzero local lateral pressure $\Pi(z)$ may exist, constrained only insofar as the sum of the pressures over the thickness of the entire bilayer gives the total lateral pressure, which must be zero: $\Sigma_z \Pi(z) = 0$. While there is as yet no direct measurement of these localized lateral pressures, there is both experimental and theoretical evidence of their magnitude and distribution with respect to depth in the bilayer. For example, the curvature elastic properties (spontaneous curvature and curvature elastic moduli) of bilayers are integral moments of the pressure 'profile' (i.e. its depth-dependence) and its curvature derivatives, as discussed below. Predictions of the pressure profile have also been obtained with analytical theory (Ben-Shaul, 1995; Harris and Ben-Shaul, 1997; Cantor, 1999), and Monte Carlo (Harris and Ben-Shaul, 1997) and molecular dynamics (Xiang and Anderson, 1994) simulations for various lipid systems.

The nonzero lateral pressure profile $\Pi(z)$ arises in large part from the competition between contributions of opposite sign: a tension (negative pressure) largely localized near the interfaces, and more broadly distributed positive pressures arising predominantly from chain conformational entropy, as well as from head-group repulsions (Israelachvili et al., 1980; Ben-Shaul, 1995; Seddon and Templer, 1995; Cantor, 1997a,b, 1998, 1999; Harris and Ben-Shaul, 1997). The interfacial tension derives from the large free energy cost of contact between hydrocarbon and water at each of the two hydrophilic/hydrophobic interfaces. This contribution to the free energy is approximately proportional to the area of interfacial contact, the constant of proportionality being roughly 0.05 J/m^2 of interface (equivalently, a constant interfacial tension of 0.05 N/m = 50 dyn/cm), using a typical value for fluid hydrocarbon/water interfaces. Acting alone, this contribution would induce the bilayer to minimize the area per molecule, e.g. for saturated chains, to align the chains in their all-trans configuration. However, the chain conformational entropy, which reflects the degree of chain conformational disorder, also makes a large contribution to the free energy of the bilayer. In contrast to the interfacial free energy, the chain conformational contribution to the pressure depends sensitively on molecular area. This pressure is very large at small molecular areas (when the acyl chains are necessarily very orientationally ordered, so even a small increase in molecular area allows for a large increase in conformational freedom), but at larger areas per molecule, at which the chains are already quite conformationally disordered, the change in entropy upon lateral expansion is much smaller. (The entropy eventually goes through a maximum with increasing area, beyond which the conformational freedom of the chains is reduced.) It is important to note that upon lateral expansion, the volume occupied by the lipids changes very little, since the energetic cost of creating free volume (the increase in van der Waals energy among the hydrocarbon chains) would be enormous; rather, the bilayer thins as it expands laterally, the chains becoming increasingly bent and intertwined, thus able to sample more of the enormous number of their configurational states, while filling up all the space in the bilayer interior at roughly constant bulk density.

The free energy minimum that defines the bilayer equilibrium derives from the compromise between these opposing forces, at which the pressures arising from interfacial tension and chain conformational (and head group) interactions are just balanced. In Fig. 1, the dependence on molecular area of these two main contributions to the free energy is simplistically portrayed using a square lattice model of short (4-segment) flexible chains in a two-dimensional bilayer. While the picture does not accurately describe the details of chain rotational isomeric states, it correctly illustrates the balance between the opposing effects of varying molecular area on the interfacial and chain conformational (and head group) contributions to the free energy that occurs at constant bulk density in the hydrocarbon domain. Although this picture is very simple, it nonetheless clearly indicates that it can be misleading to de-

scribe lipid molecules as having a characteristic 'shape', e.g. conical or cylindrical. The average shape of a lipid can only indicate the depth-dependence of the volume it occupies on average, which is overwhelmingly determined by the geometric constraints of the aggregate. Furthermore, there is no obvious relation, for example, between the degree of chain orientational order (a well-defined thermodynamic equilibrium property, either as a function of bilayer depth, or of position along the acyl chains) and less well-defined concepts such as free volume or the existence of packing defects. In any case, there is no simple relation between molecular details (chain unsaturation, etc.) and the distribution of lateral pressures in the bilayer, as has been suggested (Epand, 1998).

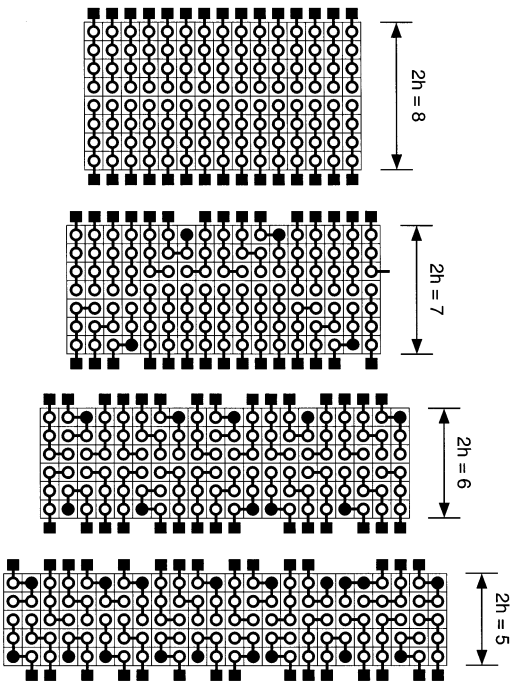

Fig. 1. A two-dimensional lattice model of short (4-segment) flexible chains is used to illustrate the dependence of chain conformational and interfacial contributions to the free energy as a function of molecular area (or equivalently, bilayer thickness) under the constraint of constant bulk density (one segment per site). The circles represent chain segments, filled circles indicating segments in contact with water; squares represent head groups. In the top panel, the bilayer thickness ($2h = 8$, in units of lattice site lengths) is twice the extended chain length, and thus requires all the chains to be vertically oriented, so there is zero conformational entropy, nor is there any contact between chain segments and water. As the bilayer thickness is incrementally reduced to five site lengths, the bilayer expanding laterally to maintain constant bulk density, the chain conformational disorder increases (although not uniformly as a function of depth in the bilayer) while the number of (energetically costly) water-exposed chain segments increases, in proportion to the area per lipid. Note that these changes in the contributions to the free energy all occur in the absence of any 'packing defects' or 'free volume'.

A rough estimate of the magnitude of the lateral pressures in the bilayer interior is readily obtained (Cantor, 1997a,b, 1998) by noting that the sum of the lateral stresses distributed over the hydrophobic interior of the fluid bilayer (ignoring head group contributions) must balance the pair of interfacial tensions at the interfaces. Using $\gamma = 0.05$ N/m as the tension of each interface, the chains must generate an opposing lateral pressure of equal magnitude distributed over the hydrophobic interior of the bilayer, of thickness ($2h$) somewhere in the range of 25–30Å. The lateral pressure density (force per unit area), i.e. the lateral pressure (force per unit length) per unit thickness of the bilayer is thus *on average* roughly $2\gamma/2h \approx 350$ atm. While other contributions to the lateral pressure will alter this number somewhat, it nonetheless provides a measure of the magnitude of the lateral pressure densities acting upon an inclusion such as a protein or peptide aggregate that passes through the bilayer interior. The actual pressure profile will be nonuniform, in a manner that depends sensitively upon the molecular composition of the bilayer (Harris and Ben-Shaul, 1997; Cantor, 1999).

3. Protein conformational equilibrium

The transmembrane domain of each of the conformational states ($s = r, t, \ldots$) of a membrane protein is characterized by a cross-sectional area $A_r, A_t, \ldots$, that depends (in general) on the depth z within the bilayer. In a conformational change $t \rightarrow r$, the cross-sectional area of the protein at depth z thus changes by an amount $\Delta A(z) = A_r(z) - A_t(z)$. As with the bilayer, it is convenient to imagine dividing the transmembrane region of the protein into thin slices of thickness δz. Let $2h$

be the thickness of the bilayer (assumed for simplicity to be comprised of identical monolayers), and let $z=0$ be the location of the bilayer midplane. For a slice centered at depth z, the mechanical work accompanying the protein conformational change depends both on the volume change of the protein $\Delta V(z)=\delta z\ \Delta A(z)$, and the lateral pressure density of the bilayer $p(z)=\Pi(z)/\delta z$ against which it either expands ($\Delta V(z)>0$) or contracts ($\Delta V(z)<0$) laterally. The total work is obtained by summing over the entire bilayer ($-h\le z\le h$):

$$W=-\Sigma_z p(z)\Delta V(z)=-\Sigma_z \Pi(z)\ \Delta A(z)$$
$$\approx -\int_{-h}^{h} p(z)\ \Delta A(z)\ \mathrm{d}z \qquad (1)$$

Define $p_0(z)$ to be the pressure profile of a bilayer having some standard lipid composition (i.e. the 'standard state' of the bilayer) in which the protein conformational equilibrium is given by $K_0=[r]_0/[t]_0$. A change in bilayer composition will result in a different pressure profile $p(z)$, and conformational equilibrium $K=[r]/[t]$. The relation between K and K_0 is readily obtained by a simple thermodynamic argument (Cantor, 1997a,b, 1998). Equating the chemical potentials of the two conformational states, $\mu_r=\mu_t$, both in the standard and in the altered bilayer environments, and assuming that $\Delta A(z)$ is independent of $\Delta p(z)$ gives $K=K_0\,\mathrm{e}^{-\alpha}$, where

$$\alpha=(k_\mathrm{B}T)^{-1}\int_{-h}^{h}\Delta p(z)\ \Delta A(z)\ \mathrm{d}z \qquad (2)$$

and $\Delta p(z)=p(z)-p_0(z)$. A positive value of α corresponds to protein inhibition ($K<K_0$), while a negative value corresponds to activation ($K>K_0$). The shift in conformational equilibrium required to have a significant inhibitory or excitatory effect on protein function (for example, as a shift in a dose-response curve) will depend on the protein, and how its activity is measured. As an order-of-magnitude estimate, one might expect that a difference between K and K_0 by a factor of at least two, i.e. $|\alpha|\ge\ln(2)$, will significantly alter protein activity. Note that since the relative change in protein conformational equilibrium K/K_0 depends exponentially on α, it is thus very sensitive both to the redistribution of lateral pressures resulting from altered bilayer composition and on the change in the cross-sectional area profile of the protein.

4. General description of shape changes of transmembrane domains

It is useful to consider a general functional description of $A_s(z)$. For smoothly varying cross-sectional area profiles, $A_s(z)$ can be expressed as an expansion in powers of z: $A_s=A_s(0)+a_{1,s}z+a_{2,s}z^2+\ldots$, for $-h\le z\le h$. However, this does not allow for a sharp kink in the profile (i.e. a discontinuity in $\mathrm{d}A/\mathrm{d}z$) as might result if the bundle were comprised of sharply bent helices. Assuming for simplicity that any kinks occur near the bilayer midplane, then a general expression that accounts for such kinks is obtained by redefining each $a_{j,s}$ as a pair of coefficients $a_{j,s}^{\pm}$ with different values on the two sides of the bilayer, i.e. $a_{j,s}^{\pm}=a_{j,s}^{+}$ for $z>0$, and $a_{j,s}^{\pm}=a_{j,s}^{-}$ for $z<0$, and thus $A_s=A_s(0)+a_{1,s}^{\pm}|z|+a_{2,s}^{\pm}z^2+\ldots$

A change in conformational state ($t\rightarrow r$) is then given by the general expression $\Delta A(z)=\Delta A(0)+\Delta a_1^{\pm}|z|+\Delta a_2^{\pm}z^2+\ldots$, with $\Delta a_j^{\pm}=a_{j,r}^{\pm}-a_{j,t}^{\pm}$. If the lipid bilayer is symmetric, i.e. if the composition of the two monolayers is identical and thus $p(z)=p(-z)$, then the expression for α in Eq. (2) simplifies to:

$$\alpha=(k_\mathrm{B}T)^{-1}\Sigma_j\ \Delta a_j\ \Delta P_j \qquad (3)$$

where $\Delta a_j=\Delta a_j^{+}+\Delta a_j^{-}$, and we define, for $j\ge 1$,

$$\Delta P_j=\int_0^h z^j\ \Delta p(z)\ \mathrm{d}z \qquad (4)$$

being the difference in the jth integral moments of $p(z)$ and $p_0(z)$ for half of the bilayer. Note that ΔP_0 is identically zero (i.e. there is no $j=0$ contribution to α) since the zeroth moment of the pressure profile, the total membrane lateral pressure, is unaffected by a redistribution of pressures (and is zero in any case).

In general, it is expected that the dominant terms in the expansion of α in Eq. (3) will be the

lowest-order nonzero terms. Thus, to the extent that coupling of changes in pressure profile to changes in protein shape is the mechanism of lipid modulation of protein activity, the equilibrium is largely determined by the first and possibly the second integral moment of the pressure profile of a monolayer, i.e. of half the bilayer. As is well known (Ben-Shaul, 1995; Seddon and Templer, 1995), the first moment gives the product of the splay curvature elastic modulus k and the spontaneous curvature c_0 of the monolayer: $P_1 = \int zp(z)\,dz = kc_0$. Note that the depth z^* of the surface of inextension (the 'neutral surface' at which small curvature deformations occur at constant molecular area) of the half-bilayer is irrelevant in calculating the first moment for a monolayer under zero net lateral stress, since $P_1 = \int(z - z^*)p(z)\,dz$ is independent of z^*. The value of c_0 is important in determining the tendency of the lipid to form cylindrical aggregates such as inverted hexagonal phases, i.e. its 'nonlamellar tendency' (Gruner, 1989; Seddon and Templer, 1995). The second moment is also related to curvature elastic properties. The Gaussian curvature elastic modulus $k_G = \int(z - z^*)^2 p(z)\,dz$ is related to the second moment $P_2 = \int z^2 p(z)\,dz$ through $k_G = P_2 - 2z^*P_1$, which contributes (along with the first moment) to the tendency to form non-cylindrical curved geometries such as bicontinuous phases.

5. Special case: geometry of a cooperative tilt

In some cases, α-helices can well be approximated as fairly smooth cylinders, so that as a crude approximation, the protein transmembrane domain can be described as a bundle of rigid rods. The change in shape of the bundle may then be described using a simple geometric representation, essentially identical to the effect of taking a tight bundle of long, cylindrically-symmetric rigid rods (e.g. pencils), clasping around one end of the bundle with one hand and around the other end with the other hand, and twisting the bundle by rotating one's hands in opposite directions around the bundle axis. When the axes of the rods are all parallel and perpendicular to the bilayer plane, the bundle has uniform cross-sectional area. As the angle between each rod and the bilayer plane decreases from 90°, the bundle splays at both ends, the cross-sectional area increasing non-uniformly, to the greatest degree at greatest distance from the center of the bundle.

The shape of the envelope of a twisted bundle is readily characterized. As defined above, the z-axis is perpendicular to the bilayer, the x–y plane ($z = 0$) defines the bilayer midplane, and thus $z = +h$ and $-h$ at the two hydrophobic/hydrophilic interfaces, so the thickness of the bilayer is $2h$, presumed to be less than the length of the bundle. Of course, the lateral packing of the rods will vary with the protein, but for simplicity, it will be assumed that for a vertically-aligned bundle, i.e. when the rod axes are aligned with the z axis, the bundle is roughly circular in cross-section, so the exterior rods (i.e. on the outside of the bundle) are all at roughly the same radial distance from the center of the bundle, as shown in Fig. 2. The cross-section of the envelope of the bundle is then approximately circular (ignoring the 'bumpiness' of the rods) with area $A = \pi\xi^2$, where ξ (independent of z for a vertically-aligned bundle) represents the distance from the center of the bundle to the outside surface of an exterior rod. The cooperative tilt described above in qualitative terms is defined as follows: each rod is rotated through the same angle θ around the line in the bilayer midplane passing from the center of the bundle through the center of the rod. For this cooperative tilt, the cross section of the bundle remains (approximately) circular at all depths, but as shown in Fig. 2(c), its cross-sectional area varies quadratically with z: $A(z) = \pi\xi(z)^2 = \pi[\xi(0)^2 + z^2\tan^2\theta]$. If the r and t states of the bundle are characterized by tilt angles θ_r and θ_t, then the change in cross-sectional area is

$$\Delta A(z) = \pi z^2(\tan^2\theta_r - \tan^2\theta_t). \quad (5)$$

Note that ΔA is independent of ξ, and thus the number of rods in the bundle (i.e. its cross-sectional area) is irrelevant; ΔA depends only on tilt angle and bilayer thickness. This is of considerable importance, since the possibility of a cooperative tilt presumes that the tilting of one rod does not interfere with the tilting of the others, which is

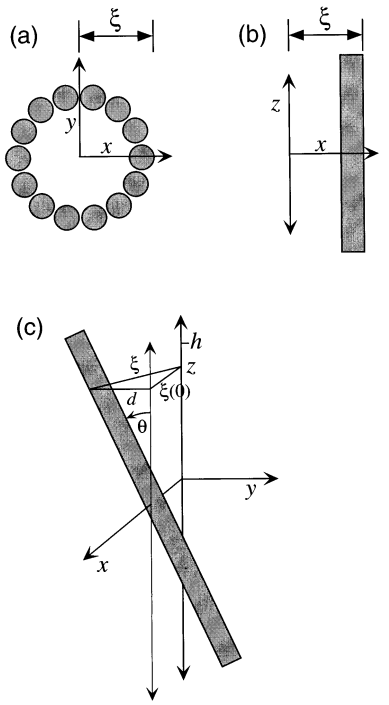

Fig. 2. (a) The exterior helices of a vertically-aligned bundle viewed in cross section of the bilayer (x–y) plane. (b) The location of an exterior helix viewed in the x–z plane, showing the distance ξ from the center of the bundle to the outside face of the helix, i.e. the radius of the envelope of the bundle. (c) An exterior helical rod rotated by an angle θ around an axis that passes through the center of the helix and the center of the bundle. The perpendicular distance from the axis of the center of the bundle to the exterior of the helix varies with bilayer depth z as $\xi(z) = [\xi(0)^2 + z^2 \tan^2 \theta]^{1/2}$.

equivalent to saying that ΔA is independent of the radius of the bundle ξ.

With the geometric result obtained above, the expression for α in Eq. (3) can be reexpressed as

$$\alpha = (2\pi/k_B T)(\tan^2 \theta_r - \tan^2 \theta_t)\Delta P_2 \quad (6)$$

where, as defined in Eq. (4), ΔP_2 is the difference in the second integral moments of $p(z)$ and $p_0(z)$. A redistribution in pressures toward the bilayer interior results in $\Delta P_2 < 0$ and thus shifts the protein equilibrium toward the state with greater tilt angle, while a pressure redistribution toward the interfaces gives $\Delta P_2 > 0$, and the equilibrium shifts toward the conformation with smaller tilt angle.

6. Equilibria involving bent helices

The cooperative tilt mechanism described above is only one of many possible rearrangements of the transmembrane domain that might occur during a protein conformational change. For example, there are many proteins (and peptides) with transmembrane helices that are sharply bent, often resulting from the presence of a proline residue, typically located near the center of the bilayer. For a collection of such kinked helices arranged around the outside of a bundle, each bent by an angle ϕ_0, the envelope of the bundle can be approximated as a pair of truncated conical sections, one in each monolayer, joined at the bilayer midplane. The angles ϕ^+ and ϕ^- between the side of each conical section and the bilayer normal clearly depend on the orientation of the bent helix with respect to rotation around the bilayer normal, relative to the center of the bundle. For example, for symmetrically disposed helices, ϕ can vary between $-\phi_0/2$ and $\phi_0/2$, the extremal values (having respectively the largest and smallest cross-sectional area at the bilayer midplane) occuring when the plane defined by the bent helix passes through the center of the bundle. For the general (asymmetric) case, the radius ξ of a circular cross-sectional slice of the bundle can be roughly approximated as linear in $|z|$, so for each state s, $\xi_s(z>0) \approx \xi_s(0) + |z| \tan \phi_s^+$, and $\xi_s(z<0) \approx \xi_s(0) + |z| \tan \phi_s^-$. Since $A_s(z) \approx \pi[\xi_s(z)]^2$, substituting this approximation for the area profile into Eq. (3) gives

$$\alpha \approx (\pi/k_B T) \times \{2\Delta[(\tan \phi^+ + \tan \phi^-)\xi(0)]\Delta P_1 + \Delta[\tan^2 \phi^+ + \tan^2 \phi^-]\Delta P_2\} \quad (7)$$

The second term in Eq. (7) is typically much smaller than the first, so in general, α is expected to depend predominantly on the first moment of the pressure profile, i.e. on kc_0.

7. Calculations

Unfortunately, a quantitatively accurate description of the change in shape of the transmem-

brane region has not yet been determined for the conformational transition of any intrinsic membrane protein. However, for the generic shape changes discussed above, α varies with $\Delta p(z)$ only through ΔP_1 and ΔP_2, so useful predictions can nonetheless be made even in the absence of specific information on $\Delta A(z)$. Simply stated, bilayers of different composition that are predicted to have similar values of P_1 and P_2 should have similar effects on the activity of a given membrane protein, if coupling of the redistribution of lateral pressure with a change in the cross-sectional area profile is the dominant mechanism by which lipid composition affects protein activity.

In recent work (Cantor, 1999), calculations of the pressure profile $p(z)$ have been performed using a statistical thermodynamic lattice model for bilayers of a wide range of lipid composition, incorporating variations in acyl chain length, position and degree of unsaturation and addition of cholesterol and small interfacially-active solutes. The results for P_1 and P_2 reported here use the predictions of $p(z)$ given therein, to which the reader is referred for a description of the theoretical methodology, choice of parameters and other calculational details, and plots of the predicted pressure profiles. [These predictions of $p(z)$ were presented for systems with head group repulsions of varying characteristic strength. However, for present purposes of calculating moments of the pressure profile, only systems without head group repulsions are considered, since the results depend sensitively on the value of z at which the repulsions are estimated to act, which is not well established for phospholipids.] Values of P_1 and P_2 are presented in Table 1 for bilayers of different acyl chain composition. For saturated chains, increasing chain length results in a significant decrease in both P_1 and P_2, largely because the distribution of lateral pressures broadens with the accompanying increase in bilayer thickness, which thus increases the distance from the center of the bilayer at which the interfacial tension acts. The effect of incorporation of a single *cis*-double bond clearly depends very strongly on the position of the unsaturation. As is evident by comparison of results for 18:0 and 18:1Δ_m for varying m, addition of a *cis*-double bond near the methyl tail has very little effect on the pressure profile, but as the double bond is placed closer to the head group, there is a marked shift of pressures toward the interface, so the values of P_1 and P_2 increase significantly. The highly unsaturated lipids 18:3$\Delta_{6,9,12}$, 20:4$\Delta_{5,8,11,14}$, and 22:6$\Delta_{4,7,10,13,16,19}$ have similar, very high values of P_1 and P_2, again resulting from a large redistribution of pressure from the bilayer interior toward the aqueous interface, as compared to saturated chains. Values of P_1 and P_2 for 1:1 mixtures of lipids are much more influenced by the properties of the more unsaturated chains in the mixture; with respect to $p(z)$, 16:0/18:1Δ_9 mixtures act much more like 18:1Δ_9 than like 16:0 bilayers, the asymmetry being even more pronounced for 16:0/22:6 mixtures. Addition of cholesterol is predicted always to decrease P_1 and P_2, but the magnitude of its effect varies strongly with the host lipid. Saturated chains are most strongly affected, while highly

Table 1
Predicted first and second moments of the pressure profile, for bilayers of varying acyl chain composition[a]

Chain composition	P_1/k_BT (Å^{-1})	P_2/k_BT
14:0	−1.74	−29.4
16:0	−1.84	−34.1
16:0 + 10% chol	−2.18	−42.1
18:0	−1.93	−38.7
20:0	−2.01	−43.2
18:1Δ_{15}	−1.84	−36.5
18:1Δ_{12}	−1.78	−33.8
18:1Δ_9	−1.57	−27.9
18:1Δ_9 + 10% chol	−1.72	−31.4
18:1Δ_6	−1.43	−25.4
18:1Δ_3	−1.29	−22.8
18:2$\Delta_{9,12}$	−1.55	−26.6
18:3$\Delta_{9,12,15}$	−1.51	−26.1
18:3$\Delta_{6,9,12}$	−1.32	−20.9
20:4$\Delta_{5,8,11,14}$	−1.31	−21.7
22:6$\Delta_{4,7,10,13,16,19}$	−1.30	−22.5
22:6 + 10% chol	−1.34	−23.6
16:0/18:1Δ_9	−1.65	−29.4
16:0/18:1Δ_9 + 10% chol	−1.84	−34.1
16:0/20:4$\Delta_{5,8,11,14}$	−1.39	−23.4
16:0/22:6$\Delta_{4,7,10,13,16,19}$	−1.36	−23.4
16:0/22:6 + 10% chol	−1.43	−25.0
16:0/22:5$\Delta_{4,7,10,13,16}$	−1.36	−23.5
16:0/22:5$\Delta_{7,10,13,16,19}$	−1.53	−27.9

[a] The mixed acyl chain compositions are equimolar.

unsaturated chains experience the smallest decrease. In all cases, the effect of adding cholesterol on P_1 and P_2 is found to be quite nonlinear in cholesterol mole fraction x_{chol} (data not shown), the magnitude $|dP_1/dx_{chol}|$ increasing significantly with increasing x_{chol}.

For the highly unsaturated chains, such as $22{:}6\Delta_{4,7,10,13,16,19}$, the effect of removing one of the six double bonds, either nearest the methyl end or nearest the head group was also examined. In a 1:1 mixture with 16:0 chains, replacing 22:6 with $22{:}5\Delta_{4,7,10,13,16}$ had almost no effect, while eliminating the double bond near the head group (replacing 22:6 with $22{:}5\Delta_{7,10,13,16,19}$) significantly decreased both P_1 and P_2. This is of interest in light of the work of Pawloski and Salem (1995), who measured the acyl chain composition of the brain and retina of cats maintained on a diet providing minimal levels of essential (polyunsaturated) fatty acids. They found that cats with prolonged exposure to alcohol, and thus with decreased capacity to generate 22:6 fatty acids, compensated with elevated levels of $22{:}5\Delta_{4,7,10,13,16}$, but not $22{:}5\Delta_{7,10,13,16,19}$ in the brain and retinal membranes. These results are consistent with a homeostatic mechanism designed to maintain the pressure profile, presumably to regulate proper membrane protein activity.

In Table 2 are reported the predicted shifts in the first moment of the pressure profile, ΔP_1, upon addition of 1 mol% of various double-chain lipids, cholesterol and single-chain cosurfactants (e.g. *n*-alkanols) of varying chain length *n* into a 'standard' lipid bilayer, arbitrarily chosen to be comprised of 16:0 acyl chains. For such small changes in composition, the predicted ΔP_1 are additive and linear in the change, to good approximation, allowing the relative 'potency' of each added component to be compared for the chosen host lipid. The effect of a singly-unsaturated chain is much greater for the (unnatural) $18{:}1\Delta_3$ chain, with its double bond close to the head group, than for $18{:}1\Delta_9$. Increasing unsaturation causes a much greater effect, only if the unsaturation begins close to the head group. Cholesterol is predicted to have an effect nearly equal in magnitude (but opposite in sign) to that of the highly unsaturated chains. For *n*-alkanols, the effect on the pressure profile decreases rapidly with increasing alkanol length. Tethering a short alcohol at one end to the interface requires its volume to be localized near the interface, where the pressure increases markedly, compensated by a decrease in pressure spread through the bilayer interior. The spatial localization, and thus the magnitude of the effect decreases rapidly as the alkanol approaches the length of the lipid chains. Finally, it is important to note that all these predicted changes depend strongly on the composition of the bilayer to which the additions are made. For example, as mentioned above, starting with a pure 16:0 bilayer, the rate of increase in P_1 with added 22:6 diminishes with increasing concentration of 22:6, while the rate of decrease in P_1 with added cholesterol grows rapidly with increasing cholesterol mole fraction.

Table 2
Predicted changes in the first and second moments of the pressure profile, upon addition of 1 mol% of double-chain lipids, cholesterol, and single-chain cosurfactants (e.g. *n*-alkanols) of chain length *n*, for a host bilayer comprised of 16:0 lipids

Additive	$\Delta P_1/k_BT$ (Å^{-1})	$\Delta P_2/k_BT$
18:0	−0.0005	−0.047
$18{:}1\Delta_3$	0.0127	0.287
$18{:}1\Delta_9$	0.0069	0.179
$18{:}2\Delta_{9,12}$	0.0103	0.291
$18{:}3\Delta_{9,12,15}$	0.0113	0.313
$18{:}3\Delta_{6,9,12}$	0.0240	0.619
$20{:}4\Delta_{5,8,11,14}$	0.0316	0.793
$22{:}6\Delta_{4,7,10,13,16,19}$	0.0402	0.978
Cholesterol	−0.0254	−0.630
Cosurfactant (*n*-alkanol):		
$n=3$	0.0074	0.170
$n=6$	0.0042	0.095
$n=9$	0.0023	0.057
$n=12$	0.0011	0.035

8. Sensitivity of equilibria to lipid compositional changes

It is important to estimate whether these simple geometric descriptions of protein conformational equilibria are sensitive enough to redistributions

in the pressure profile resulting from small changes in lipid composition to result in significant changes in protein activity. As a representative example, assume that a significant modulation in activity results from a change by a factor of two (up or down) in the protein conformational equilibrium K, i.e. for which $|\alpha| = \ln(2)$. Consider first the collective tilt model, in which α is determined by θ_t and θ_r. Because α varies as $\tan^2\theta$, much smaller pressure redistributions are required to obtain the same α for a given change in tilt angle, $\Delta\theta = \theta_r - \theta_t$, at larger angles. Somewhat arbitrarily, consider $\tan^2\theta_r - \tan^2\theta_t = 0.05$, corresponding, for example, to a tilt of only $\Delta\theta \approx 3°$ at $\theta_t = 20°$, but of $\Delta\theta \approx 13°$ at $\theta_t = 0°$. For the bent-helix reorientation model, α depends on $\tan\phi_t$ and $\tan\phi_r$. Consider a bundle of radius $\xi(0) \approx 20$ Å, as might be representative of ligand-gated ion channel proteins, and a fairly small angular change: $\Delta(\tan\phi) \approx 0.1$, corresponding to $\Delta\phi \approx 6°$. The potency of various additives to a given standard bilayer composition is then determined by the mole fraction of the additive required to obtain $|\alpha| = \ln(2)$, i.e. either $|\Delta P_2|/k_B T \approx 2.2$ (from Eq. (6), for the collective tilt model) or $|\Delta P_1|/k_B T \approx 0.028$ Å^{-1} (from Eq. (7), for the model involving reorientation of bent helices.) While these are only order-of-magnitude (and somewhat conservative) approximations, from the results of calculations presented in Tables 1 and 2, it is clear that even fairly small compositional changes (e.g. addition of only a few mol% cholesterol or a highly unsaturated lipid to a 16:0 bilayer) are enough to obtain a significant change in protein conformational equilibrium, even for the small shape changes chosen as examples.

9. Discussion

The calculations of $p(z)$ involve many approximations and simplifications, involving the use of a mean-field (random mixing) approximation in describing the environment of each chain, a crude lattice model to describe chain conformational states, a constant interfacial tension, etc (Cantor, 1999). However, even with these approximations it is expected that the predicted trends with regard to variations in chain length, unsaturation, addition of cholesterol and small interfacially-active solutes, etc. are at least qualitatively accurate. But perhaps of greater concern is the neglect of the effect of the protein on the pressure profile. While this may be a good approximation at very low protein concentrations, it is certainly not true at the very high protein concentrations that exist in biological membranes; it is well known that the presence of peptides and proteins can significantly alter the phase behavior of lipid bilayers (Epand, 1996, 1998). Recently, the modulation of bilayer properties by proteins, with particular interest in phase stability and curvature elastic properties, has been carefully examined by May and Ben-Shaul (1999). Ultimately, the predicted influence of proteins on the pressure profile must be included in a full treatment of lipid modulation of protein function.

The specific predictions for P_1 and P_2 for varying chain unsaturation, cholesterol content, and addition of alcohols of varying chain length correlate well, for example, with the activity of rhodopsin (Mitchell et al., 1992; Litman and Mitchell, 1996; Mitchell et al., 1996; Brown, 1997). Litman and Mitchell (1996) report a sixfold increase in the Meta-II/Meta-I equilibrium K_{eq} in going from di-14:0 to 16:0/20:4 PC lipids, with much smaller increases from 16:0/20:4 to 16:0/22:6 to di-20:4 to di-22:6 PC lipids (a total of a factor of two). The calculations yield qualitatively similar results, predicting a large increase in P_1 in going from 14:0 to 16:0/20:4 lipids, and smaller increments in the same order: 16:0/20:4 to 16:0/22:6 to di-20:4 to di-22:6. Mitchell et al. (1992) observed a significant decrease in this equilibrium with added cholesterol, consistent with the predicted decrease in P_1, while K_{eq} was found to increase with added short-chain alkanols (Mitchell et al., 1996), the effect diminishing rapidly with increasing alkanol chain length, consistent with the predictions reported in Table 2. Interestingly, they find that for sufficiently long alkanol chains, the shift in

equilibrium changes direction, i.e. K_{eq} decreases upon addition of long-chain alkanols. This may be due to the large head-group repulsions characteristic of PC head groups. Calculations that incorporate strong repulsions among the lipid head groups, but not between the alkanol and the lipids (results not shown) predict a significant reduction in the values of ΔP_1 (as compared to results in the absence of head-group repulsions) for all alkanol chain lengths, eventually becoming negative for the longer *n*-alkanols. Short alkanols have little effect on the average separation of the repulsive PC head groups, while the longer alkanol chains increase the average separation considerably. For sufficiently long chains, the resulting decrease in the large positive lateral pressure in the head group region is more than enough to compensate for the shift in the pressure profile in the acyl chain region toward the aqueous interface, which also rapidly decreases in significance as the alkanol chain length approaches that of the lipid chains.

The basic thermodynamic results for α in Eqs. (3), (6) and (7), respectively for the general case, and for the more specific changes involving a cooperative tilt in bundles of either straight or sharply bent helices, are all predicted to depend on the first and/or second moments of the lateral pressure profile, which in turn uniquely determine key curvature elastic properties of the lipid monolayer. The coupling between lipid-dependent redistributions of lateral pressures and changes in the cross-sectional area profile accompanying protein conformational transitions may thus reveal the physical underpinnings of the strong correlation between the nonlamellar tendency of the lipids comprising the bilayer and modulation of activity of membrane proteins.

Acknowledgements

I am extremely grateful for the warm hospitality and nourishing scientific environment provided by Professor Ole Mouritsen and the members of the MemPhys group at the Technical University of Denmark.

References

Ben-Shaul, A., 1995. Molecular theory of chain packing, elasticity and lipid–protein interaction in lipid bilayers. In: Dynamics of Membranes. Elsevier/North Holland, Amsterdam, pp. 359–401.

Bienvenüe, A., Sainte Marie, J., 1994. Modulation of protein function by lipids. Curr. Topics Membr. 40, 319–354.

Brown, M.F., 1997. Influence of nonlamellar-forming lipids on rhodopsin. Curr. Topics Memb. 44, 285–356.

Cantor, R.S., 1997a. Lateral pressures in cell membranes: a mechanism for modulation of protein function. J. Phys. Chem. 101, 1723–1725.

Cantor, R.S., 1997b. The lateral pressure profile in membranes: a physical mechanism of general anesthesia. Biochemistry 36, 2339–2344.

Cantor, R.S., 1998. The lateral pressure profile in membranes: a physical mechanism of general anesthesia. Tox. Lett. 100–101, 451–458.

Cantor, R.S., 1999. Lipid composition and the lateral pressure profile in bilayers. Biophys. J. 76, 2625–2639.

Chu, B., Dopico, A.M., Lemos, J.R., Treistman, S.N., 1998. Ethanol potentiation of calcium-activated potassium channels reconstituted into planar lipid bilayers. Mol. Pharm. 54, 397–406.

Epand, R.M. (Ed.), 1996. The properties and biological roles of non-lamellar forming lipids. Chem. Phys. Lipids 81(2), 101–264.

Epand, R.M., 1998. Lipid polymorphism and protein–lipid interactions. Biochim. Biophys. Acta 1376, 353–368.

Gruner, S.M., 1989. Stability of lyotropic phases with curved interfaces. J. Phys. Chem. 93, 7562–7570.

Harris, D., Ben-Shaul, A., 1997. Conformational chain statistics in a model lipid bilayer: comparison between mean-field and Monte Carlo calculations. J. Chem. Phys. 106, 1609–1619.

Israelachvili, J., Marcelja, S., Horn, R., 1980. Physical principles of membrane organization. Q. Rev. Biophys. 13, 121–200.

Litman, B.J., Mitchell, D.C., 1996. A role for phospholipid polyunsaturation in modulating membrane protein function. Lipids 31, S193–197.

May, S., Ben-Shaul, A., 1999. Molecular theory of lipid–protein interaction and the L_a–H_{II} transition. Biophys. J. 76, 751–767.

Mitchell, D.C., Straume, M., Litman, B.J., 1992. Role of *sn*-1-saturated, *sn*-2-polyunsaturated phospholipids in control of membrane receptor conformational equilibrium: Effects of cholesterol and acyl chain unsaturation on the metarhodopsin I↔metarhodopsin II equilibrium. Biochemistry 31, 662–670.

Mitchell, D.C., Lawrence, J.T.R., Litman, B.J., 1996. Primary alcohols modulate the activation of the G protein-coupled receptor rhodopsin by a lipid-mediated mechanism. J. Biol. Chem. 271, 19033–19036.

Pawloski, R.J., Salem, N.J. Jr., 1995. Ethanol exposure causes a decrease in docosahexaenoic acid and an increase in docosapentaenoic acid in feline brains and retinas. Am. J. Clin. Nutr. 61, 1284–1289.

Rankin, S.E., Addona, G.H., Kloczewiak, M.A., Bugge, B., Miller, K.W., 1997. The cholesterol dependence of activation and fast desensitization of the nicotinic acetylcholine receptor. Biophys. J. 73, 2446–2455.

Seddon, J.M., 1990. Structure of the inverted hexagonal (H_{II}) phase, and non-lamellar phase transitions of lipids. Biochim. Biophys. Acta 1031, 1–69.

Seddon, J.M., Templer, R.H., 1995. Polymorphism of lipid-water systems. In: Lipowsky, R., Sackmann, E. (Eds.), Structure and Dynamics of Membranes. Elsevier/North Holland, Amsterdam, pp. 97–160.

Unwin, N., 1993. Nicotinic acetylcholine receptor at 9 Å resolution. J. Mol. Biol. 229, 1101–1124.

Unwin, N., 1995. Acetylcholine receptor channel imaged in the open state. Nature 373, 37–43.

Xiang, T.-X., Anderson, B.D., 1994. Molecular distribution in interphases: statistical mechanical theory combined with molecular dynamics simulations of a model lipid bilayer. Biophys. J. 66, 561–573.