Spring Constants for Channel-Induced Lipid Bilayer Deformations Estimates Using Gramicidin Channels

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ABSTRACT Hydrophobic interactions between a bilayer and its embedded membrane proteins couple protein conformational changes to changes in the packing of the surrounding lipids. The energetic cost of a protein conformational change therefore includes a contribution from the associated bilayer deformation energy (ΔG^0_{def}), which provides a mechanism for how membrane protein function depends on the bilayer material properties. Theoretical studies based on an elastic liquid-crystal model of the bilayer deformation show that ΔG^0_{def} should be quantifiable by a phenomenological linear spring model, in which the bilayer mechanical characteristics are lumped into a single spring constant. The spring constant scales with the protein radius, meaning that one can use suitable reporter proteins for *in situ* measurements of the spring constant and thereby evaluate quantitatively the ΔG^0_{def} associated with protein conformational changes. Gramicidin channels can be used as such reporter proteins because the channels form by the transmembrane assembly of two nonconducting monomers. The monomer↔dimer reaction thus constitutes a well characterized conformational transition, and it should be possible to determine the phenomenological spring constant describing the channel-induced bilayer deformation by examining how ΔG^0_{def} varies as a function of a mismatch between the hydrophobic channel length and the unperturbed bilayer thickness. We show this is possible by analyzing experimental studies on the relation between bilayer thickness and gramicidin channel duration. The spring constant in nominally hydrocarbon-free bilayers agrees well with estimates based on a continuum analysis of inclusion-induced bilayer deformations using independently measured material constants.

INTRODUCTION

The hydrophobic coupling between integral membrane proteins and the bilayer acyl chains (Owicki et al., 1978) causes protein conformational changes that involve the protein-bilayer interface (Unwin and Ennis, 1984; Unwin et al., 1988) to perturb the structure of the surrounding bilayer (Israelachvili, 1977) (Fig. 1). (See Mouritsen and Andersen (1998) for recent overviews of membrane structure and function.) The energetic cost (ΔG^0_{tot}) associated with a protein conformational change thus will include a contribution from the associated bilayer deformation energy (ΔG^0_{def}), and the bilayer material constants are among the determinants of protein conformational preference and function (Owicki et al., 1978; Mouritsen and Bloom, 1984; Gruner, 1985, 1991; Huang, 1986; Andersen et al., 1992; Keller et al., 1993; Brown, 1994; Lundbæk and Andersen, 1994; Lundbæk et al., 1996, 1997).

The bilayer material constants vary as a function of the bilayer lipid composition (Evans and Needham, 1987); the associated changes in ΔG^0_{def} may provide a mechanism for the control of protein function by the membrane lipid composition. Changes in bilayer composition, for example, affect the distribution among different functional states of integral membrane proteins (Brown, 1994; Chang et al., 1995a,b; Lundbæk et al., 1996) as well as their catalytic activity (Caffrey and Feigenson, 1981; Johannsson et al., 1981; Navarro et al., 1984; Starling et al., 1995). The changes in protein function usually occur in the absence of specific lipid-protein interactions (e.g., Devaux and Seigneuret, 1985; Bienvenüe and Marie, 1994), and they can be induced pharmacologically by compounds that alter the bilayer's phase propensity (e.g., McCallum and Epand, 1995).

The quantitative contribution of ΔG^0_{def} to ΔG^0_{tot} remains poorly understood. Studies using model peptides suggest that ΔG^0_{def} can be substantial (Huang, 1986; Keller et al., 1993; Lundbæk and Andersen, 1994; Lundbæk et al., 1996, 1997). The extrapolation of these results to integral membrane protein function has been difficult, however. First, the theory of inclusion-induced bilayer deformations (Huang, 1986; Helfrich and Jakobsson, 1990; Dan et al., 1994; Nielsen et al., 1998) is complex, as ΔG^0_{def} is the sum of three contributions: a compression-expansion component, a splay-distortion component, and an interfacial energy/surface tension component (Fig. 1). The (relative) magnitudes of these contributions to ΔG^0_{def} vary as a function of the underlying material constants, as well as the choice of boundary conditions at the protein/lipid interface (Nielsen et al., 1998). Second, it is not clear whether the quadratic approximation used in elastic (liquid crystal) theories of bilayer behavior (Helfrich, 1973; Huang, 1986) is valid when the curvature radii are comparable to the membrane thickness or whether macroscopic material constants can be used to describe such systems (Helfrich, 1981). This latter

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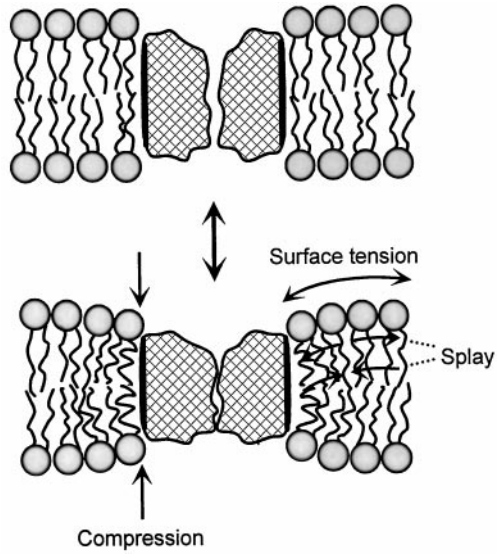

FIGURE 1 Hydrophobic coupling between an integral membrane protein and the bilayer acyl chains causes protein conformational changes that involve the protein-bilayer interface, to alter the structure of the immediately surrounding bilayer. The total free energy of the protein conformational change (ΔG^0_{tot}) will include a contribution from the associated bilayer deformation energy (ΔG^0_{def}).

concern is accentuated because the contributions to ΔG^0_{def} are interdependent: a change in the splay-distortion modulus will change not only the splay-distortion component but also the compression-expansion component of ΔG^0_{def}, and vice versa (Nielsen et al., 1998).

A potentially important simplifying feature was identified by Nielsen et al. (1998), who showed that ΔG^0_{def} in many cases can be quantified using a linear spring description, where the bilayer material constants are lumped together in a single phenomenological spring constant whose magnitude scales with the dimensions of the imbedded membrane inclusion (protein). In this article we use results of previous experimental studies (Kolb and Bamberg, 1977; Elliott et al., 1983) to show that ΔG^0_{def} indeed can be described by a linear spring model. We further provide numerical estimates for the phenomenological spring constants in hydrocarbon-containing and hydrocarbon-free bilayers. The spring constant in nominally hydrocarbon-free bilayers is in good agreement with predictions based on macroscopic material constants (Nielsen et al., 1998), which provides justification for the use of elastic liquid crystal theories to describe protein-induced bilayer deformations.

GRAMICIDIN CHANNELS AS FORCE TRANSDUCERS

Gramicidin (gA) channels are miniproteins, formed by the transmembrane dimerization of two monomers, one from each monolayer of a bilayer (O'Connell et al., 1990) (Fig. 2). The nonconducting monomers are inserted into monolayers as $\beta^{6.3}$ helices (He et al., 1994).

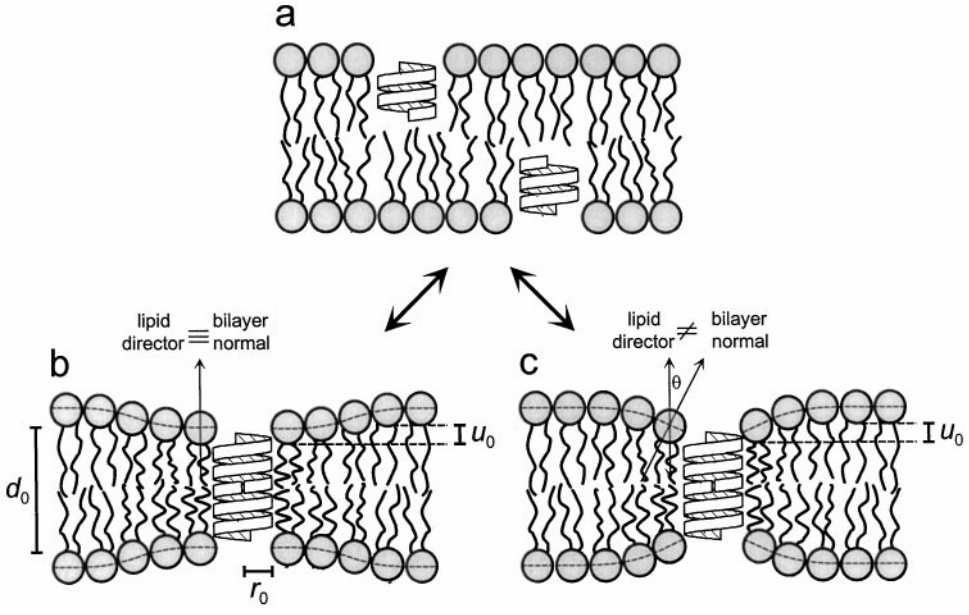

FIGURE 2 Gramicidin channel formation and bilayer deformation. (*a*) Two isolated monomers, one in each monolayer. (*b*) Schematic illustration of the bilayer deformation associated with gA channel formation when the acyl chain packing at the channel/lipid boundary is constrained, that is, when the director of the lipids in contact with the channel is parallel to the channel exterior and the bilayer normal. (*c*) Schematic illustration of the bilayer deformation associated with gA channel formation when the acyl chain packing at the channel/lipid boundary is free, when the details of lipid packing at the channel/lipid boundary can be ignored, and when the director is not parallel to the bilayer normal. d_0 denotes the equilibrium thickness of the unperturbed bilayer, u_0 the deformation depth in each monolayer, r_0 the channel radius, and θ the angle between the local bilayer normal and the lipid director representing the preferred orientation of the lipid acyl chains (the bilayer normal and lipid director are shown as vectors directed away from the bilayer). The interrupted curves through the head groups (in *b* and *c*) depict the general shape of the membrane deformation.

There is no evidence for specific interactions between gA channels and their host bilayer (Providence et al., 1995; Girshman et al., 1997). Furthermore, the helical pitch of the gA channel is not affected by lipid phase transitions or acyl chain length (Katsaras et al., 1992), meaning that the channel length can be considered invariant with respect to the extent of the bilayer deformation (but see Mobashery et al., 1997). When the length of the channel's hydrophobic exterior differs from the bilayer hydrophobic thickness, channel formation will perturb the surrounding bilayer. This bilayer deformation has an associated ΔG^0_{def}. Channel dissociation is associated with a corresponding bilayer relaxation and a ΔG^0_{def} of equal magnitude but opposite sign. The average channel lifetime (τ) therefore depends on the magnitude of ΔG^0_{def}, and gA channels can be used as force transducers (Lundbæk et al., 1996; Andersen et al., 1998) to evaluate the membrane deformation energy.

The relation between the depth of the deformation in each monolayer (u_0) and the bilayer deformation energy ($\Delta G^0_{def}(u_0)$) is described using the linear spring approximation (Nielsen et al., 1998):

$$\Delta G^0_{def}(u_0) = H(2u_0)^2, \quad (1)$$

where H is a phenomenological spring constant describing the channel-induced membrane deformation. The magnitude of H is determined by the bilayer area-compression and splay-distortion (or bending) moduli, as well as the channel radius (r_0) and the boundary condition chosen to describe the lipid packing at the bilayer/channel interface (Nielsen et al., 1998), specifically the angle θ between the bilayer normal and the lipid director (denoting the preferred orientation of the acyl chains) adjacent to the channel (Fig. 2).

To proceed, we make the standard assumption of strong hydrophobic coupling between the channel and the bilayer core, meaning that the bilayer deformation, $2u_0$, is given by

$$2u_0 = d_0 - l, \quad (2)$$

where d_0 is the equilibrium thickness of the unperturbed bilayer core and l is the hydrophobic length of the channel exterior (Fig. 2); $l \approx 2.2$ nm (Elliot et al., 1983), meaning that l usually is less than d_0. This is important, as the use of gA channels as molecular force transducers depends on $l < d_0$, i.e., on the bilayer's tending to pull the channels apart.

When the channel dissociates, the monomers separate a distance δ before the transition state is reached. The dissociation rate constant (k_{dis}) can be described as

$$\ln\{k_{dis}\} = -\ln\{\tau\} = -\Delta G^{\ddagger}/RT - \ln\{\tau_0\}, \quad (3)$$

where $\Delta G^{\ddagger}$ is the activation energy for channel dissociation, R is the gas constant, T is the temperature in Kelvin, and $1/\tau_0$ is a frequency factor for the reaction. $\Delta G^{\ddagger}$ can be described as

$$\Delta G^{\ddagger} = \Delta G^{\ddagger}_{int} + \Delta\Delta G^0_{def} = \Delta G^{\ddagger}_{int} + H((2u_0 - \delta)^2 - (2u_0)^2)$$
$$= \Delta G^{\ddagger}_{int} - H(4u_0 - \delta)\delta, \quad (4)$$

where $\Delta G^{\ddagger}_{int}$ is the intrinsic activation energy and $\Delta\Delta G^0_{def}$ is the difference in bilayer deformation energy for deformations of $2u_0$ and $2u_0 - \delta$. Combining Eqs. 3 and 4:

$$\ln\{k_{dis}\} = -(\Delta G^{\ddagger}_{int} - H(4u_0 - \delta)\delta)/RT - \ln\{\tau_0\}, \quad (5)$$

or

$$d(\ln\{k_{dis}\})/du_0 = 4H\delta/RT. \quad (6)$$

That is, $\ln\{k_{dis}\}$ is a linear function of u_0 (or d_0), which allows for a determination of H (assuming δ is known).

ANALYSIS OF EXPERIMENTAL RESULTS

Fig. 3 shows the experimental dependence of τ on d_0 for gA channels in monoglyceride bilayers (Kolb and Bamberg, 1977; Elliott et al., 1983). The results are shown as $-\ln\{\tau\}$ ($= \ln\{k_{dis}\}$) versus d_0. d_0 was varied by changing the acyl chain length of the monoglyceride using monopalmitolein (16:1), monoolein (18:1), monoeicosenoin (20:1), monoerucin (22:1), or mononervonin (24:1). (In very thick bilayers ($C_{24:1}$/*n*-hexadecane, $d_0 = 6.9$ nm) the gA single-channel conductance is reduced more than 10-fold compared with thinner bilayers, suggesting that the channel structure is

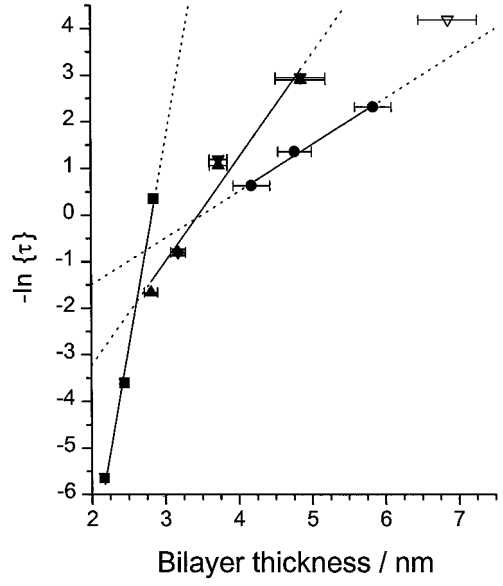

FIGURE 3 The dependence of τ on d_0. Results plotted as $-\ln\{\tau\}$ ($= \ln\{k_{dis}\}$) vs. d_0. The experiments were done using either squalene in 0.5 M KCl (■) ($C_{16:1}$, $\tau = 286$ s; $C_{18:1}$, $\tau = 37$ s; $C_{20:1}$, $\tau = 0.7$ s (Elliott et al., 1983)); *n*-hexadecane in 1 M NaCl (▲) ($C_{16:1}$, $\tau = 5.3$ s; $C_{18:1}$, $\tau = 2.2$ s; $C_{20:1}$, $\tau = 0.34$ s; $C_{22:1}$, $\tau = 0.05$ s), or 1 M CsCl (▼) ($C_{18:1}$, $\tau = 2.2$ s; $C_{20:1}$, $\tau = 0.30$ s; $C_{22:1}$, $\tau = 0.05$ s; $C_{24:1}$, $\tau = 0.015$ s, plotted as open symbol (Kolb and Bamberg, 1977)); *n*-decane in 1 M NaCl (●) ($C_{16:1}$, $\tau = 0.53$ s; $C_{18:1}$, $\tau = 0.26$ s; $C_{22:1}$, $\tau = 0.10$ s (Kolb and Bamberg, 1977)). In bilayers formed using squalene, τ was determined from single-channel experiments. In *n*-hexadecane and *n*-decane-containing bilayers, τ was determined from autocorrelation experiments. The lines through the three set of data points denote least-squares fits of straight lines to the data. The bilayer hydrophobic thickness was determined from capacitance measurements (Elliott et al., 1983; Benz et al., 1975). The horizontal lines for the data in decane- and hexadecane-containing bilayers denote ± 1 SD.

altered (Kolb and Bamberg, 1977). Thickness-related changes in gA channel structure do, in fact, occur in very thick bilayers (Mobashery et al., 1997); we therefore exclude the $C_{24:1}$/*n*-hexadecane results from the quantitative analysis. We further note that strong hydrophobic coupling, meaning that Eq. 2 is obeyed, is expected to fail for monoglyceride/*n*-hexadecane bilayers with $d_0 > 6.0$ nm (see Discussion).) The hydrocarbon solvent was either *n*-decane, *n*-hexadecane, or squalene. Bilayers formed using squalene are virtually hydrocarbon-free (Simon et al., 1977; White, 1978). For all three systems, $\ln\{k_{dis}\}$ (or $-\ln\{\tau\}$) is a linear function of d_0 over bilayer thickness changes that vary between ~0.7 nm (relative change, ~25%) for monoglyceride/squalene bilayers, ~2.0 nm (relative change, ~40%) for monoglyceride/*n*-hexadecane bilayers, and ~1.7 nm (relative change, ~30%) for monoglyceride/*n*-decane bilayers. (The relative changes in u_0 are even larger: >10-fold in monoglyceride/squalene bilayers, ~3-fold in monoglyceride/*n*-hexadecane bilayers, and ~2-fold in monoglyceride/*n*-decane bilayers.) Each line is determined by only three (or four) data points, but the large relative variations in d_0 (and u_0) allow us to conclude that the relation between k_{dis} (and thus ΔG^0_{def}) and u_0 can be described by a linear spring model over a (surprisingly) large range of u_0 (or d_0).

The slopes of the $\ln\{k_{dis}\}$ versus d_0 plots vary with the hydrocarbon solvent: $d(\ln\{k_{dis}\})/dd_0$ in bilayers formed from monoglyceride/squalene solutions is four- or ninefold larger than in monoglyceride/*n*-hexadecane bilayers or monoglyceride/*n*-decane bilayers (Table 1). Using Eq. 6, H can be estimated knowing δ, the distance the monomers has moved apart before reaching the transition state for channel dissociation. The transition state reflects the breaking of some of the hydrogen bonds that stabilize the dimer. Removing a single hydrogen bond at the join between the monomers decreases the channel stability 500-fold (Durkin et al., 1993). The alternating L-D sequence of gA (Sarges and Witkop, 1965), however, means that the monomers can be connected only by two, four, or six hydrogen bonds, as the two monomers rotate relative to each other; we therefore assume the transition state is reached when two hydrogen bonds are broken, i.e., when the monomers have moved 0.16 nm apart. The ensuing estimates of H are summarized in Table 1.

DISCUSSION

The present analysis shows that the dependence of gA channel lifetime on bilayer thickness can be described by a phenomenological elastic spring model, which is applicable to both solvent-containing and solvent-free bilayers, over a quite large range of thickness variations. gA channels form by the transmembrane association of two monomers, which causes channel formation to be associated with a well defined change in bilayer thickness (when the channel length is less than the bilayer thickness). gA channels therefore should be suitable for quantitative *in situ* estimates of the bilayer deformation energy associated with a change in the match between bilayer thickness and the hydrophobic length of an integral membrane protein (cf. Gruner, 1991).

TABLE 1 Spring constants for the gA channel/monoacylglyceride bilayer system

Solvent	$d(\ln\{k_{dis}\})/dd_0$ (nm^{-1})	H (kJ/(mol nm^2))
Squalene	8.9 ± 0.7	69 ± 6
n-Hexadecane	2.2 ± 0.2	17 ± 2
n-Decane	1.0 ± 0.1	8 ± 1

Results are given as mean ± SEM.

We first compare the magnitude of the spring constant in nominally hydrocarbon-free bilayers with predictions based on the theory of elastic liquid crystal deformations using macroscopic, continuum values for the material moduli. We then show that the assumption of strong hydrophobic coupling should be valid under the conditions used to determine the spring constant. We finally comment on previous attempts to analyze ΔG^0_{def} associated with a gA channel-induced deformation of hydrocarbon-containing bilayers.

Our estimate for the spring constant for the solvent-free gA/monoglyceride system, 69 ± 6 kJ/(mol nm^2), is independent of the channel's hydrophobic length because the slope of the $\ln\{k_{dis}\}$ versus d_0 relation is independent of the channel length (Eq. 6). The magnitude of H, however, depends on our choice of δ (Eq. 6), which we take to be 0.16 nm based on the alternating L-D sequence and experimental results on the effects of removing a single residue at the join between the monomers that form the channel (Durkin et al., 1993). The value of δ is unlikely to be larger than 0.16 nm, but could be smaller, in which case H would be larger than indicated in Table 1. Given this uncertainty, the estimates for H compares well with predictions based on a continuum theory of liquid crystal deformations, as detailed below.

When the area compression-expansion modulus (K_a) and the splay-distortion modulus (K_c) for the bilayer are known, one can predict H using the following expression, which can be derived from the scaling relations in Nielsen et al. (1998, p. 1975):

$$H = H^* \left(\frac{K_a}{K_a^*}\right)^{\nu} \left(\frac{K_c}{K_c^*}\right)^{\mu}, \tag{7}$$

where H^*, K_a^*, and K_c^* denote a reference parameter set and ν and μ are empirically determined coefficients. $K_a^* = 142.5$ pN/nm and $K_c^* = 28.5$ pN nm; the magnitude of H^*, ν, and μ depends on the choice of boundary conditions at the channel/bilayer interface (Nielsen et al., 1998). When the boundary condition is constrained, that is when the director of the lipids in contact with the channel is parallel to the channel exterior and the local bilayer normal (cf. Fig. 2 *b*), $H^* = 63.6$ kJ/(mol nm^2), $\nu = 0.667$, and $\mu = 0.334$; when the boundary condition is free, when the details of lipid packing at the channel/lipid boundary can be ignored and the lipid director is tilted relative to the bilayer normal (cf. Fig. 2 *c*), $H^* = 21.7$ kJ/(mol nm^2), $\nu = 0.717$, and $\mu = 0.287$. (Nielsen et al. (1998) should be consulted for a

discussion of the physical significance of the different boundary conditions.)

For pure monoolein bilayers, K_c is estimated to be 36 ± 4 pN nm (Chung and Caffrey, 1994). K_a has been estimated to be 140 ± 50 pN/nm for nominally hydrocarbon-free monoolein/squalene bilayers (White, 1978; Hladky and Gruen, 1982) and 210 ± 20 pN/nm for monoolein bilayers formed from pentane (Alvarez and Latorre, 1978). (The uncertainties in K_a were estimated using Monte Carlo methods (Alper and Gelb, 1990), assigning a 30% uncertainty to the electrocompression coefficient reported by White (1978).) Using these values for K_a and K_c, and approximating the gA channel as having a cylindrical shape, H is predicted to be between 68 ± 15 kJ/(mol nm^2) and 89 ± 6 kJ/(mol nm^2) if the boundary conditions were constrained (Fig. 2 *b*), and between 23 ± 6 kJ/(mol nm^2) and 31 ± 2 kJ/(mol nm^2) if the boundary conditions were free (Fig. 2 *c*). The experimental estimate for H is in good agreement with predictions based on the constrained boundary condition, and two- to threefold larger than predictions based on the relaxed boundary condition. (The theoretical predictions for H depend on the gA channel radius, which is known only with some uncertainty (cf. Woolf and Roux, 1996; Table 2). Our predictions were based on a channel radius (r_0) of 1 nm, which could be an overestimate by up to 0.2 nm. Such an overestimate of r_0 would entail that the predicted H would be too large, by 10% or more, which would only strengthen the agreement between the experimental estimate and the predictions based on the constrained boundary conditions.) If δ were less than 0.16 nm, the discrepancy between the experimental estimate for H and the prediction(s) based on the relaxed boundary conditions would be even larger.

Considering the number of parameters involved when predicting ΔG^0_{def} (or H) using the theory of liquid-crystal deformations (Huang, 1986; Nielsen et al., 1998), the agreement between the observed and predicted H (for the constrained boundary condition) could be due to a fortuitous cancellation of errors. Although that possibility cannot be excluded, we consider the agreement to provide considerable support for using the theory of liquid crystal elastic deformations to describe membrane protein-induced perturbations of lipid bilayers (even though the extension to biological membranes may be complicated by their heterogeneous, asymmetric lipid composition). With that proviso, the agreement between our estimate for H in nominally hydrocarbon-free monoglyceride/squalene membranes and the prediction based on the constrained boundary conditions indicates that the lipid organization at the protein/lipid interface (in hydrocarbon-free bilayers) should be described using the constrained boundary condition, in agreement with the conclusion of Huang (1986).

In hydrocarbon-containing bilayers, the free boundary conditions should prevail, as the lipid packing problem at the protein/bilayer interface will be reduced because the hydrocarbon can fill any void created at the protein/lipid interface when the angle between the lipid director and the protein surface differs from zero (cf. Fig. 2 *c*). Hydrocarbons thus exert a similar effect on protein/bilayer interactions as they do on bilayer↔nonlamellar phase transitions in pure lipids (Kirk and Gruner, 1985). In addition, for either boundary condition, the compression and splay contributions to ΔG^0_{def} are reduced because the hydrocarbon can be squeezed out from between the acyl chains, which reduces H further, to below predictions based on the free boundary conditions (in a hydrocarbon-free bilayer), as is observed (cf. Table 1).

An implicit assumption in the above analysis, and all previous work on membrane protein/lipid bilayer interactions, is that the hydrophobic coupling between the channel's exterior surface and the bilayer is sufficiently strong to ensure that Eq. 2 is valid. The range of membrane thickness variations that were used in the experiments of Kolb and Bamberg (1977) and Elliott et al. (1983) is so large, however, that it is necessary to validate the assumption of strong hydrophobic coupling. Following Andersen et al. (1998), strong hydrophobic coupling should prevail, and Eq. 2 remain valid, as long as

$$\frac{d(\Delta G^0_{def}(u_0))}{d(2u_0)} = 4Hu_0 < \Delta G^*_{hydrophobic}, \qquad (8)$$

where $\Delta G^*_{hydrophobic}$ denotes the hydrophobic energy associated with exposing a unit length (1 nm) long segment of the channel exterior (or bilayer acyl chains) to water. The hydrophobic energy is ~20 kJ/(mol nm^2) (Sharp et al., 1991) and the gA channel radius is 1 nm, so $\Delta G^*_{hydrophobic} \approx$ 125 kJ/(mol nm). Strong hydrophobic coupling therefore should prevail as long as $2u_0 \leq 0.9$ nm or $d_0 \leq 3.1$ nm (in monoglyceride/squalene bilayers), $2u_0 \leq 3.6$ nm or $d_0 \leq 5.8$ nm (in monoglyceride/*n*-hexadecane bilayers), and $2u_0 \leq 8.1$ nm or $d_0 \leq 10.3$ nm (in monoglyceride/*n*-decane bilayers). Comparing these limits to the data in Fig. 3, the assumption of strong hydrophobic coupling should be valid, except for mononervonin/hexadecane bilayers, which were excluded from the quantitative analysis.

Previously, Helfrich and Jakobsson (1990) evaluated the deformation energy in hydrocarbon-containing bilayers. In their analysis the ΔG^0_{def} associated with gA channel formation in hydrocarbon-containing bilayers was evaluated using a sandwich approximation in which the hydrocarbon was assumed to be localized in a separate phase in the membrane interior. A bilayer-compressing force therefore would work on two springs in series: one spring denotes thinning the bilayer to the hydrocarbon-free thickness and is characterized by an area compression-expansion modulus K^1_a; another spring denotes compression of the hydrocarbon-free bilayer and is characterized by an area compression-expansion coefficient $K^2_a \cdot K^2_a$ is expected to be ~$1000 \cdot K^1_a$ (Helfrich and Jakobsson, 1990). Thus, when the bilayer thickness is varied by changing the acyl chain length, thinning the bilayer to the hydrocarbon-free thickness would be an almost constant minor contribution to ΔG^0_{def} (Helfrich and Jakobsson, 1990; Durkin et al., 1993). The hydrocarbon should not influence the membrane thickness dependence of

ΔG^0_{def}. The solvent dependence of H (Fig. 3 and Table 1) does not support the sandwich approximation. This finding could have been predicted from the results of McIntosh et al. (1980), who found that the longer hydrocarbons are interdigitated parallel to the acyl chains and not positioned in the middle of the bilayer.

In conclusion, gA channels can be used to measure the phenomenological spring constant that describes the membrane deformation energy associated with an imposed change in bilayer lipid packing. The spring constant in nominally hydrocarbon-free bilayers is in good agreement with the value predicted using an elastic liquid-crystal theory of bilayer deformations, which provides support for the use of macroscopic material constants when evaluating membrane protein-bilayer interactions and for the neglect of the higher-order terms in the expression for the membrane deformation energy (Helfrich, 1973, 1981). Moreover, the energetics of channel-bilayer interactions can be described by a linear spring model even in hydrocarbon-containing bilayers, which suggests that gA channels can be used to evaluate the mechanical properties of bilayers of arbitrary chemical composition (including the bilayer component of biological membranes). Thus, because the spring constant scales as an approximately linear function of protein radius (Nielsen et al., 1998), one should be able to use experimentally determined spring constants to evaluate the bilayer deformation energy associated with protein conformational changes in many different membrane environments.

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