Gramicidin Channels: Molecular Force Transducers in Lipid Bilayers

O. S. Andersen, C. Nielsen, A. M. Maer
J. A. Lundbæk, M. Goulian *and* R. E. Koeppe II

Abstract

The thermodynamic need to maximize hydrophobic interactions between integral membrane proteins and their host bilayer serves as one of the major guiding principles in models of biological membranes. These hydrophobic interactions govern not only the folding and membrane insertion of the proteins, they also affect membrane protein function. The control of protein function is due to the hydrophobic coupling between the membrane-spanning part of integral membrane proteins and the surrounding bilayer core. This coupling causes protein conformation changes that involve the protein/lipid interface to perturb the surrounding bilayer. The elastic membrane deformation energy associated with a protein conformational change thus will contribute to the overall free energy difference between different protein conformations. The importance of these membrane elastic deformations can be evaluated using ion channels, where measurements of the channel-mediated current allows for a direct measure of the (equilibrium) distribution among different protein conformations.

O. S. Andersen, C. Nielsen, A. M. Maer
Department of Physiology and Biophysics
Cornell University Medical College
New York, NY 10021, USA
e-mail: sparre@mail.med.cornell.edu

M. Goulian
Center for Studies in Physics and Biology
The Rockefeller University
New York, NY 10021, USA

J. A. Lundbæk
Neuropharmacology Unit
Novo-Nordisk A/S
DK-2760 Måløv, Denmark

R. E. Koeppe II
Department of Chemistry and Biochemistry
University of Arkansas
Fayetteville, AR 72701, USA

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Introduction

The fluid-mosaic membrane model evolved from thermodynamic considerations about the organization of the main membrane components - phospholipids, cholesterol, and proteins (Singer and Nicolson, 1972). The guiding principle underlying the development of the model, the need to maximize hydrophobic and hydrophilic interactions, has served as a central organizing theme in all subsequent work. The main feature of the model, that the lipids are organized in a liquid-crystalline

bilayer in which integral membrane proteins are imbedded, had a similar immediate appeal. A weakness of the model was that the lipid bilayer component was assumed to be a passive entity only - a permeability barrier that separated the extracellular and intracellular aqueous phases. This point of view was strengthened by numerous studies on the permeability of lipid bilayers to small polar solutes (e.g. Walter and Gutknecht, 1986), which showed that the lipid bilayer could be approximated as being a ~5 nm thin sheet of liquid hydrocarbon. The possible importance of geometric packing criteria (Israelachvili, 1977), and the material properties of the lipid bilayer (Helfrich, 1973; Evans and Hochmuth, 1978), for lipid- protein interactions and the lateral organization of biological membranes largely were ignored.

The failure to appreciate the significance of the liquid-crystalline organization of lipid bilayers, with the associated material properties (thickness and compression modulus, curvature and bending modulus), also has implications for attempts to understand the mechanisms underlying the control of membrane protein function by the membrane lipids. Numerous studies (e.g. Devaux and Seigneuret, 1985; Bienvenüe and Marie, 1994) have shown that membrane protein function is affected by the membrane lipid composition - and by whether the lipids are in the gel or liquid-crystalline state. The view of the lipid bilayer as a sheet of liquid hydrocarbon led to the notion of bilayer fluidity as an important determinant of protein function.

The limitations of this notion were exposed by Lee (1991), who pointed out that a change in bilayer fluidity alone cannot explain a shift in the conformational preference of integral membrane proteins. If not fluidity, what then?

Membrane Protein Conformation Changes and Bilayer Perturbations

Structural studies on membrane proteins show that membrane protein function may involve changes in protein structure that affect the protein/lipid interface (Unwin and Ennis, 1984; Unwin et al., 1988). The hydrophobic coupling between the membrane-spanning domain of integral membrane proteins and the bilayer core will cause such a protein conformational change to perturb the structure of the immediately surrounding bilayer (Fig. 1). The free energy difference (ΔG^0_{tot}) between two protein conformations is the sum of contributions from the protein *per se* (ΔG^0_{prot}) and terms that arise from the protein's interactions with the environment, which include the deformation energy (ΔG^0_{def}) arising from the bilayer perturbation. This separation of the total free energy into intrinsic and extrinsic terms may be problematic, but the distinction helps to differentiate energetic contributions that arise from intramolecular rearrangements in the protein interior from the energetic cost of the intermolecular reorganization that occurs at the protein surface.

ΔG^0_{def} varies as a function of the material properties of the lipid bilayer, which, in principle, provides the means for control of the protein conformational preference and function by the bilayer lipid composition. Bilayer deformations (bilayer compression and bending) can be described using the theory of liquid-crystal deformations (Helfrich, 1973). This notion was further developed by Huang (1986) to describe the energetics of inclusion-induced membrane deformations (monolayer bending and compression). In such models of elastic membrane deformations, ΔG^0_{def} will be a function of the membrane deformation (u). When the energetic penalty associated with exposing hydrophobic groups to water is much larger than the

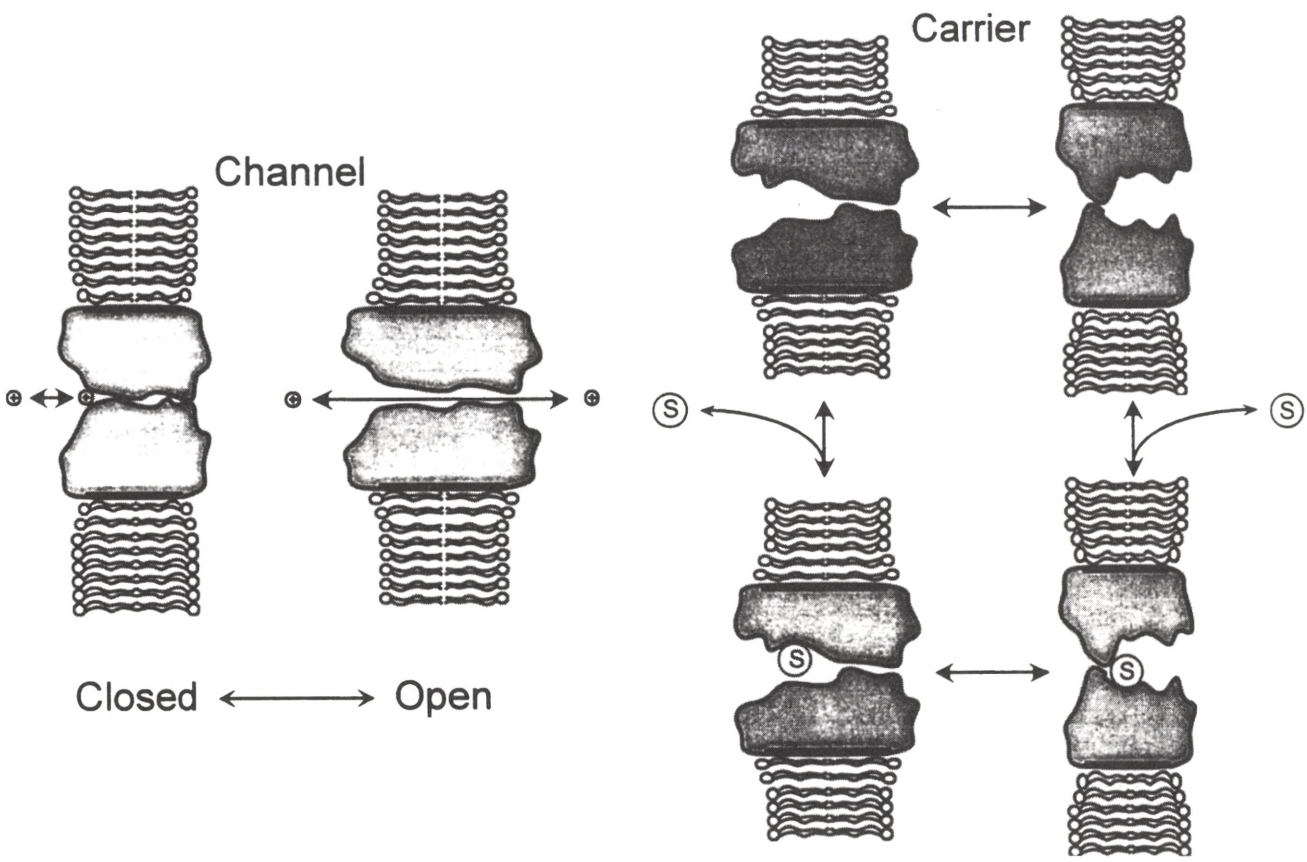

Figure 1. Solute transfer by membrane-spanning channels (left) and conformational carriers (right).

membrane deformation energy, i.e. in the limit of strong hydrophobic coupling , then u will be equal to the difference between the membrane hydrophobic thickness (d) and the protein's hydrophobic exterior length (l). In elastic membrane models, ΔG^0_{def} can in many cases be described as a quadratic function of u (Lundbæk et al., 1996; Nielsen et al., 1998):

$$\Delta G^0_{\text{def}} = A \cdot u^2 \qquad (1)$$

where A is a phenomenological spring constant associated with the membrane deformation. Is the bilayer deformation energy of sufficient magnitude to affect the protein conformational preference and function? To address this question it is necessary to have quantitative measurements that probe how the lipid bilayer affects structurally well-defined conformational transitions in membrane proteins (Gruner, 1991).

The energetic coupling between proteins and bilayers can affect the function of all imbedded proteins. There is, however, a fundamental difference between the way in which membrane-spanning channels and conformational carriers catalyze the transmembrane transfer of selected solutes (Fig. 1), which has implications for how the function of channels and carriers is affected by the lipid bilayer. In channels, the control of function arises from conformational changes between non-conducting (closed) and conducting (open) states. The catalytic event (the transfer of a solute/ion across the membrane) is uncoupled from such large-scale protein conformational changes. One therefore can use the channel-mediated ionic current to monitor directly the distribution between non-conducting (closed) and conducting (open)

channel states (conformations). In carriers, the catalytic events are inextricably coupled to protein conformation changes. A change in the equilibrium constant between the major conformers (the binding site exposed toward the left or the right in Fig. 1) will affect the rate constants for both the forward and the backward transitions (usually in opposite directions). This complicates attempts to understand how a change in bilayer material properties will affect the carrier function. The turnover rate, for example, may be a non-monotonic function of $\Delta G^0_{\rm def}$. Channels, therefore, offer advantages not enjoyed by the carriers for attempts to elucidate the basis for membrane control of protein function. (Similar advantages are offered by membrane-spanning receptors, where one likewise can monitor the equilibrium distribution between different conformations, see e.g. Brown (1994)).

Ion Channels as Tools to Study Protein-Membrane Interactions

Among ion permeable channels, the gramicidin A (gA) monomer↔dimer equilibrium, associated with the formation of membrane-spanning gA channels, constitutes a reasonably well-defined structural transition in a membrane inclusion (Figure 2). Standard gA channels are miniproteins formed by the transmembrane assembly (O'Connell et al., 1990) of two $\beta^{6.3}$-helical monomers (He et al., 1994), that join at their formyl-NH-termini to form the conducting channels (see Andersen and Koeppe, 1992; Killian, 1992, and Koeppe and Andersen, 1996, for reviews). Most, if not all, membrane-spanning gA dimers are conducting channels (Veatch et al., 1975), and there is no evidence for specific interactions between gA channels and their host bilayer (Providence et al., 1995; Girshman et al., 1997). These properties make gA suitable for investigating the bilayer mechanical properties.

The gA dimerization constant is $K_D = [D]/[M]^2$, where $[D]$ and $[M]$ denote the surface densities of gA dimers and monomers. Assuming that $\Delta G^0_{\rm def}$ is the only extrinsic contribution to $\Delta G^0_{\rm tot}$,

$$\begin{aligned} K_D &= \frac{[D]}{[M]^2} = \exp\{-\Delta G^0_{\rm tot}/kT\} \\ &= \exp\{-(\Delta G^0_{\rm prot} + \Delta G^0_{\rm def})/kT\} \\ &= K_D^{\rm prot} \cdot \exp\{-\Delta G^0_{\rm def}/kT\} \end{aligned} \quad (2)$$

where k is Boltzmann's constant, T the temperature in Kelvin, and $K_D^{\rm prot} = \exp\{-\Delta G^0_{\rm prot}/kT\}$, Rewriting Eq. 2 gives

$$\Delta G^0_{\rm def} = kT \cdot \ln\left\{\frac{[D]}{K_D^{\rm prot} \cdot [M]^2}\right\}. \quad (3)$$

The gA channel-associated membrane conductance $G = [D] \cdot g$, where g is the single-channel conductance, and Eq. 3 becomes

$$\Delta G^0_{\rm def} = kT \cdot \ln\left\{\frac{G/g}{K_D^{\rm prot} \cdot [M]^2}\right\} \quad (4)$$

which relates bilayer energetics and electrophysiological measurements. When $K_{\rm D}^{\rm prot}$ is unknown, Eq. 4 can be used to measure changes in $\Delta G^0_{\rm def}$ ($\Delta\Delta G^0_{\rm def}$) in the limit when $[D] << [M]$ (Lundbæk and Andersen, 1994; Lundbæk et al., 1997):

$$\Delta\Delta G^0_{\rm def} = -kT \cdot \ln\left\{\frac{G_{\rm exptl}/g_{\rm exptl}}{G_{\rm cntrl}/g_{\rm cntrl}}\right\}, \quad (5)$$

where the subscripts denote the experimental and control situations, respectively. It is thus possible to show that relatively modest modifications of the bilayer properties can change $\Delta G^0_{\rm def}$ by 10-15 kJ/mole (Lundbæk and Andersen, 1994; Lundbæk et al., 1997) - indicating that the bilayer deformation energy may be of sufficient magnitude to affect protein function.

Rather than $\Delta G^0_{\rm def}$, one can measure the disjoining force the bilayer imposes on the membrane-spanning gA dimers, which affects both the association (k_1) and dissociation (k_{-1}) rate constants. k_{-1} is of primary interest because $k_{-1} = 1/\tau$,

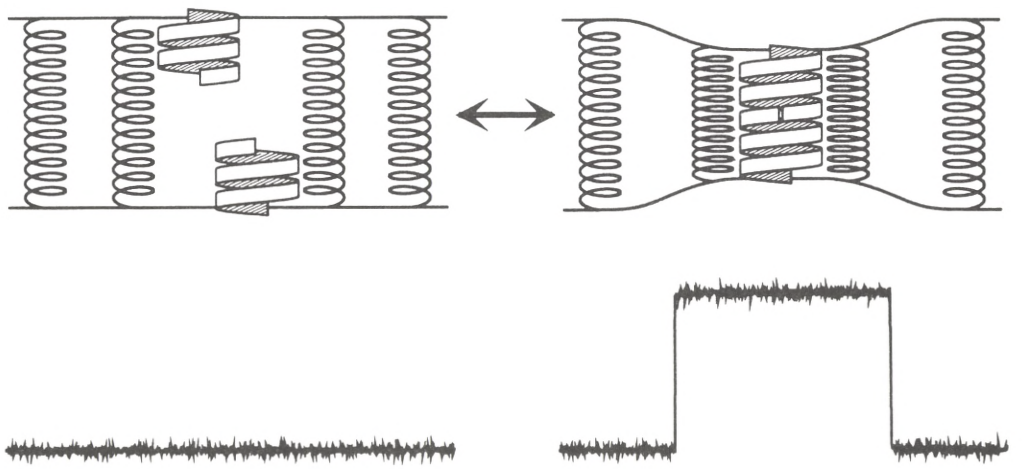

Figure 2. The gA monomer↔dimer reaction can be observed electrophysiologically. Top: schematic representation of the gramicidin monomer↔dimer equilibrium and the membrane perturbation that is associated with channel formation. Bottom: the current signal associated with channel formation/dissociation.

where τ is the average dimer (channel) lifetime, which is directly measurable

$$k_{-1} = \frac{1}{\tau_0} \cdot \exp\{-\Delta G^{\ddagger}/kT\}, \qquad (6)$$

where $\Delta G^{\ddagger}$ is the activation energy for dimer dissociation and $1/\tau_0$ is a frequency factor (in Eyring's Transition State Theory $1/\tau_0 = kT/h$). The transition state for dimer dissociation occurs when the monomers move a distance δ apart, and $\Delta G^{\ddagger}$ is the sum of the intrinsic activation energy ($\Delta G^{\ddagger}_{\rm prot}$) and the difference in bilayer deformation energy ($\Delta G^{\ddagger}_{\rm def}$) for a deformation of u and $u - \delta$. Using Eq. 1,

$$\begin{aligned} \Delta G^{\ddagger} &= \Delta G^{\ddagger}_{\rm prot} + \Delta G^{\ddagger}_{\rm def} \\ &= \Delta G^{\ddagger}_{\rm prot} + A \cdot ([u-\delta]^2 - u^2) \qquad (7) \\ &= \Delta G^{\ddagger}_{\rm prot} - A \cdot (2 \cdot u - \delta) \cdot \delta \end{aligned}$$

cf. (Lundbæk et al., 1996), and

$$\begin{aligned} \tau &= \tau_{\rm prot} \cdot \exp\{\Delta G^{\ddagger}_{\rm def}/kT\} \\ &= \tau_{\rm prot} \cdot \exp\{-A \cdot (2 \cdot u - \delta) \cdot \delta/kT\}, \qquad (8) \end{aligned}$$

where $\tau_{\rm prot} = \tau_0 \cdot \exp\{\Delta G^{\ddagger}_{\rm prot}/kT\}$. Assuming that the hydrophobic coupling is sufficiently strong (that $u = d - l$), A can be determined from the variation of τ as a function of membrane thickness (J. A. Lundbæk and O. S. Andersen, manuscript in preparation). The resulting value of A is large, indicating that the bilayer deformation energy associated with a hydrophobic mismatch can affect protein function - as well as the lateral organization of proteins in the membrane (cf. Mouritsen and Bloom, 1984).

Hydrophobic Coupling - and its Limitations

The hydrophobic coupling between integral membrane proteins and their surrounding bilayer is a central element in models of membrane organization. Limitations arise, however, for at least two reasons.

First, the hydrophobic/hydrophilic boundary is fuzzy - because of the imprecise relation between the positions of C_α and the charged, or

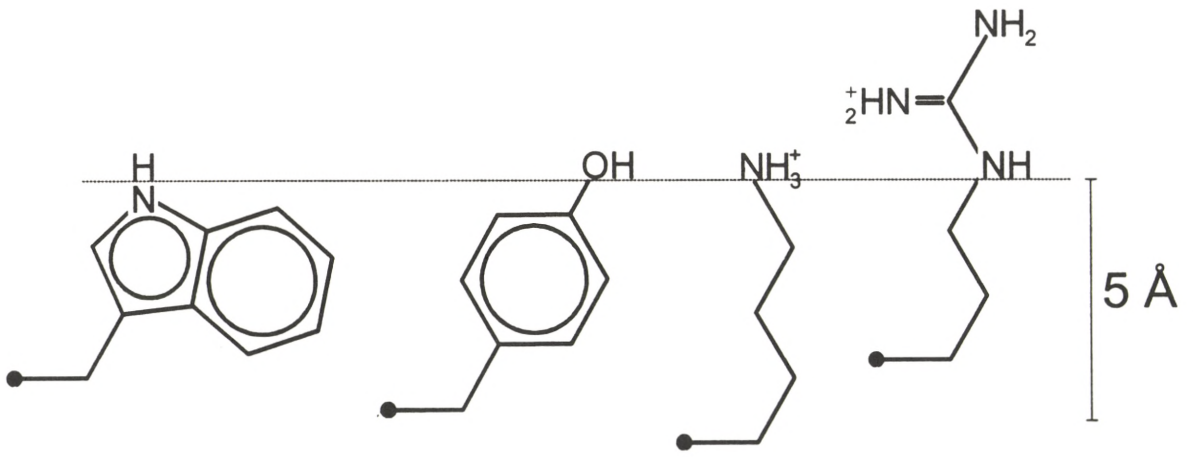

Figure 3. Amphipathic amino acids at the membrane/solution interface. The membrane/solution interface is denoted by ..... ; the C_α by •.

polar, moieties in the anchor residues that delimit membrane-spanning α-helices (Fig. 3). The $(CH_2)_3$ and $(CH_2)_4$ linkers in Arg and Lys, for example, allow the effective length of membrane-spanning α-helix to vary by several Å. Interestingly, the amphipathic aromatic residues may define the hydrophobic/hydrophilic boundary better, because the rigid ring structure provides for a better defined relation between C_α and the polar moiety. This may account for the preponderance of Trp and Tyr residues at the hydrophobic/hydrophilic boundary of integral membrane proteins (cf. Landolt-Marticorena et al., 1993).

Second, as u increases, the notion of strong hydrophobic coupling will fail ($u \neq d - l$) because ΔG^0_{def} eventually will become so large that it becomes advantageous to allow hydrophobic residues to be in direct contact with water. For example, the effective spring constant for membrane deformations adjacent to an integral membrane protein of radius 30 Å is ~4 kJ/(mol · Å^2) (C. Nielsen, M. Goulian and O. S. Andersen, in preparation). For the same protein, the hydrophobic penalty associated with a hydrophobic mismatch is ~20 kJ/(mol · Å), which means that the incremental deformation energy will exceed the incremental hydrophobic energy when $u > 2.5$ Å. Strong hydrophobic coupling therefore will fail for larger membrane deformations - and, even for small deformations, there may be some slippage.

Perspectives

The bilayer and its imbedded proteins exert reciprocal effects upon each other:

Protein Conformational Change
⇳
Bilayer Deformation Energy.

The reciprocity emphasizes the dynamic implications of the hydrophobic coupling between bilayer and proteins. That is, in addition to serving as an organizing principle for the folding of membrane proteins, the need to minimize the exposure of hydrophobic groups to water (Singer and Nicolson, 1972) also serves as an organizing principle for the regulation of protein function by the bilayer. It thus becomes important to understand how membrane lipid heterogeneity affects the dynamics and

energetics of protein conformational changes:

1. Does the local membrane lipid composition reorganize in response to membrane protein conformational changes?
2. How will such reorganization affect the lipid bilayer material properties - and the protein conformational changes?
3. What is the significance of lipid components that form non-bilayer structures; can a bilayer be too stable - e.g. because protein conformational transitions are facilitated close to the lamellar/non-lamellar phase transition for the membrane lipids?

Finally, implicit in the above is that the control of protein function by the membrane lipids *to a first approximation* is a "simple" energetic question, which can be addressed using the continuum theory of liquid-crystal deformations with minimal chemical specificity. That is, one can to a first approximation disregard the existence of numerous different membrane lipid components, and describe the bilayer as an elastic sheet. The situation thus becomes similar to that for electrified interfaces, where the Gouy-Chapman theory of the diffuse double layer serves as a major organizing principle (e.g. McLaughlin, 1989).

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