

Amphiphile regulation of ion channel function by changes in the bilayer spring constant

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Many drugs are amphiphiles that, in addition to binding to a particular target protein, adsorb to cell membrane lipid bilayers and alter intrinsic bilayer physical properties (e.g., bilayer thickness, monolayer curvature, and elastic moduli). Such changes can modulate membrane protein function by altering the energetic cost ($\Delta G_{\text{bilayer}}$) of bilayer deformations associated with protein conformational changes that involve the protein-bilayer interface. But amphiphiles have complex effects on the physical properties of lipid bilayers, meaning that the net change in $\Delta G_{\text{bilayer}}$ cannot be predicted from measurements of isolated changes in such properties. Thus, the bilayer contribution to the promiscuous regulation of membrane proteins by drugs and other amphiphiles remains unknown. To overcome this problem, we use gramicidin A (gA) channels as molecular force probes to measure the net effect of amphiphiles, at concentrations often used in biological research, on the bilayer elastic response to a change in the hydrophobic length of an embedded protein. The effects of structurally diverse amphiphiles can be described by changes in a phenomenological bilayer spring constant (H_B) that summarizes the bilayer elastic properties, as sensed by a bilayer-spanning protein. Amphiphile-induced changes in H_B , measured using gA channels of a particular length, quantitatively predict changes in lifetime for channels of a different length—as well as changes in the inactivation of voltage-dependent sodium channels in living cells. The use of gA channels as molecular force probes provides a tool for quantitative, predictive studies of bilayer-mediated regulation of membrane protein function by amphiphiles.

bilayer elasticity | hydrophobic coupling | hydrophobic matching

It long has been suspected that “membrane-active” or “membrane-stabilizing” drugs could regulate membrane protein function by partitioning into the host lipid bilayer and thereby alter its physical properties (1, 2). Indeed, numerous studies have shown that amphiphiles, including many drugs, alter lipid bilayer physical properties (for a recent review, see ref. 3). Moreover, membrane protein function involves conformational changes at the protein-bilayer boundary (4), which due to hydrophobic coupling will perturb the surrounding bilayer (Fig. 1A). The associated bilayer deformation energy ($\Delta G_{\text{bilayer}}$) contributes to the free energy of a protein conformational change (ΔG_{prot}), meaning that changes in bilayer physical properties can alter protein function by altering $\Delta G_{\text{bilayer}}$, cf. refs. 4–8. The promiscuous regulation of membrane proteins by amphiphiles therefore may be due to amphiphile-induced changes in $\Delta G_{\text{bilayer}}$. This mechanism would provide a rationale for the observed correlations between the increased hydrophobicity (or lipophilicity) of drugs and the likelihood of adverse events (9) or attrition during drug development (10, 11).

Amphiphilic molecules alter many different bilayer properties [including intrinsic curvature (12), thickness (13), and elastic moduli (14, 15)], some of which may have opposing effects on the bilayer deformation energy (4). This complicates attempts to predict even the sign of the changes in $\Delta G_{\text{bilayer}}$ (12). The problem can be overcome, however, by using gramicidin A (gA)

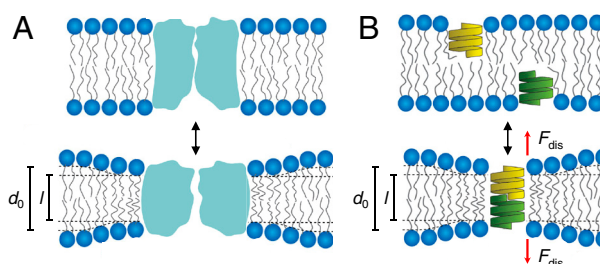
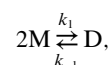


Fig. 1. Hydrophobic coupling between a bilayer-embedded protein and its host lipid bilayer. (A) A protein conformational change causes a local bilayer deformation. (B) Formation of a gA channel involves local bilayer thinning. Modified from ref. 4.

channels as probes to sense net changes in bilayer properties as experienced by a bilayer-spanning protein.

gA channels (D) formed by the transbilayer association of monomeric subunits (M) from each bilayer leaflet (Fig. 1B), and channel gating is described by



where k_1 and k_{-1} are the association and dissociation rate constants for the monomer \leftrightarrow dimer equilibrium. Channel formation in a bilayer with a hydrophobic thickness (d_0) that exceeds the channel hydrophobic length (l) involves a local bilayer deformation (16–19) with an associated deformation energy. The bilayer, in response, exerts a disjoining force (F_{dis}) on the channel, the magnitude of which is determined by the bilayer elastic properties (Fig. 1B). Amphiphiles that decrease F_{dis} will increase k_1 , which is reported as an increase in channel appearance frequency (f), and decrease k_{-1} , which is reported as an increase in channel lifetime ($\tau = 1/k_{-1}$) (4, 12).

Results and Discussion

The amphiphile Triton X-100 (TX100) increases τ , meaning that it decreases F_{dis} (12). To quantify the changes in the bilayer elastic properties, we compare the changes in τ for gA channels formed by subunits of different length and chirality; the 13-residue [des-Val¹-Gly²]gA⁻ and the 15-residue [Ala¹]gA (20) [designated gA⁻ (13) and AgA (15), respectively]. Fig. 2A shows current traces that illustrate the effects of 3 μ M TX100 on gA⁻ (13) and AgA (15) channels in diphytanoylphosphatidylcholine (DPhPC)/*n*-decane bilayers. TX100 increases f and τ for both channel types, with the larger effects on the shorter channels.

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Fig. 2B shows the concentration-dependent effects of TX100 and six other amphiphiles [capsaicin, capsazepine (12), daidzein, genistein, phloretin (21), and GsMTx4 (22)], on τ for gA channels formed by (left- or right-handed) subunits of 13 residues (τ_{13}) or 15 residues (τ_{15}). At concentrations where these amphiphiles alter membrane protein function (summarized in the original articles), they all increase τ for both channel types—and all with the larger effects on the shorter channels; cf. refs. 4, 12, 21, 22. [At the concentrations used in the gA channel experiments, the amphiphiles do not alter membrane capacitance (12, 21, 23). Thus changes in bilayer thickness cannot account for the effects.]

The changes in channel lifetimes reflect changes in activation energy for channel dissociation (ΔG^\ddagger):

$$\Delta G^\ddagger - \Delta G_{\text{cntl}}^\ddagger = \ln\{\tau/\tau_{\text{cntl}}\} \cdot k_B T, \quad [1]$$

where k_B and T are, respectively, Boltzmann's constant and the temperature in Kelvin, and “cntl” denote results in the absence of amphiphile. The larger changes in lifetime for the shorter channels relative to the longer channels mean that the changes in ΔG^\ddagger also are larger for the shorter channels than for the longer channels (Fig. 3). Moreover, because the changes in τ_{13} and τ_{15} result from the same underlying mechanism, the changes τ (in ΔG_{13}^\ddagger and ΔG_{15}^\ddagger) are likely to be correlated (5). Fig. 3A shows the changes in ΔG_{13}^\ddagger as a function of the changes in ΔG_{15}^\ddagger in experiments with DPhPC as the bilayer-forming lipid. Despite the very different structures of the amphiphiles, the changes are described by a shared linear relation with a slope: $\Delta\Delta G_{13}^\ddagger/\Delta\Delta G_{15}^\ddagger = 1.19 \pm 0.02$ (\pm SE, $r^2 > 0.99$). A similar relation is obtained using a number of other amphiphiles with dioleoylphosphatidylcholine (DOPC) as the bilayer-forming lipid. Fig. 3B shows the effects of TX100 and seven other amphiphiles [curcumin (24), docosa-

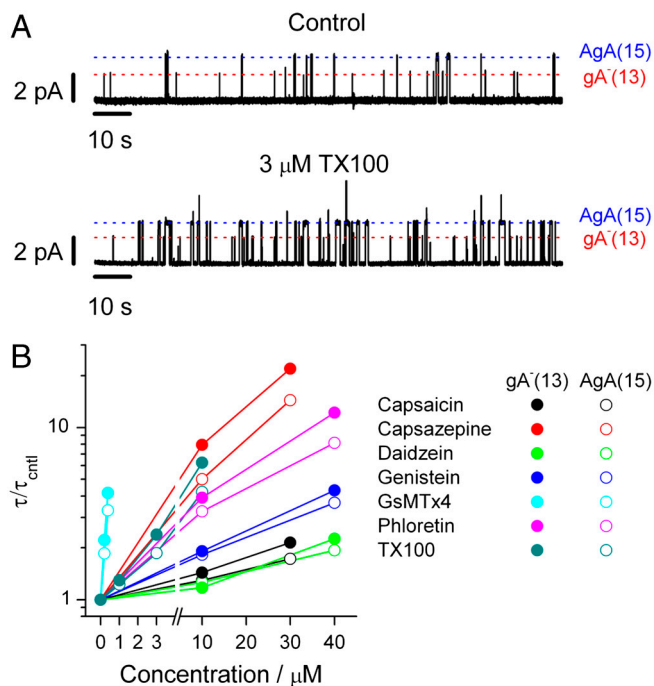


Fig. 2. Effects of amphiphiles on gA channels in DPhPC/*n*-decane bilayers. (A) Current traces before and after addition of 3 μM TX100 to both sides of a bilayer doped with gA⁻(13) and AgA(15). The red and blue lines denote the current levels for gA⁻(13) and AgA(15) channels. (B) Concentration-dependent effects of TX100, capsaicin, capsazepine, daidzein, genistein, phloretin, or GsMTx4 on lifetime of channels formed by monomeric subunits having 13 or 15 residues (except for TX100, based on results from refs. 12, 21, and 22).

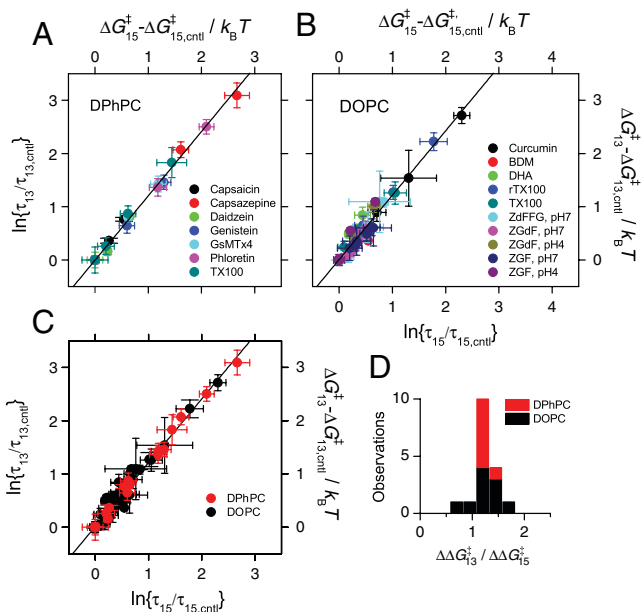


Fig. 3. Effects of amphiphiles on lifetimes of channels formed by 13-residue subunits (expressed as $\ln\{\tau/\tau_{\text{cntl}}\}$, left axis, or activation energy, $\Delta G^\ddagger - \Delta G_{\text{cntl}}^\ddagger$, right axis) vs. corresponding effects on channels formed by 15-residue subunits (bottom or top axis). (A) Effects of TX100, capsaicin, capsazepine, daidzein, genistein, phloretin, or GsMTx4 in DPhPC/*n*-decane bilayers (except for TX100, results from refs. 12, 21 and 22). A subset of the results was published previously (5). (B) Effects of TX100, curcumin, docosahexaenoic acid (DHA), 2,3-butanedione monoxime (BDM), reduced Triton X-100 (rTX100), Z-Gly-D-Phe (ZGdF), and Z-Gly-Phe (ZGF) (at pH 7 or pH 4) and Z-D-Phe-Phe-Gly (ZdFFG, at pH 7) in DOPC/*n*-decane bilayers (except for TX100, results from refs. 24–28). (C) Superimposition of results obtained using DPhPC (red) or DOPC (black). (D) Distribution of the slopes (values of $\Delta\Delta G_{13}^\ddagger/\Delta\Delta G_{15}^\ddagger$) for individual amphiphiles using DPhPC (red) or DOPC (black). Mean \pm SEM ($n \geq 3$) or \pm range ($n = 2$).

hexaenoic acid (25), 2,3-butanedione monoxime (26), reduced Triton X-100 (27); and the viral antifusion peptides Z-Gly-D-Phe, Z-Gly-Phe and Z-D-Phe-Phe-Gly (28)]. Again the results can be described by a shared linear relation: $\Delta\Delta G_{13}^\ddagger/\Delta\Delta G_{15}^\ddagger = 1.21 \pm 0.05$ (\pm SE, $r^2 = 0.95$). Indeed, the DPhPC and DOPC results can be superimposed (Fig. 3C), with the overall fit: $\Delta\Delta G_{13}^\ddagger/\Delta\Delta G_{15}^\ddagger = 1.20 \pm 0.03$ (\pm SE, $r^2 = 0.98$). Fig. 3D shows the distribution of the slopes (values of $\Delta\Delta G_{13}^\ddagger/\Delta\Delta G_{15}^\ddagger$) for the different amphiphiles.

The activation energy for gA channel dissociation is given by

$$\Delta G^\ddagger = \Delta G_{\text{channel}}^\ddagger + \Delta G_{\text{bilayer}}^\ddagger, \quad [2]$$

where $\Delta G_{\text{channel}}^\ddagger$ denotes an “intrinsic” energy cost due to loss of contributions such as hydrogen bonds that stabilize the channel dimer, whereas $\Delta G_{\text{bilayer}}^\ddagger$ denotes the change in bilayer deformation energy associated with separating the channel subunits by δ [a distance of ~ 0.16 nm (19, 29, 30)] to reach a transition state where the channel conductance is lost (Fig. 1B). A large body of work, summarized in ref. 4, has shown that the amphiphile-induced changes in τ primarily reflect changes in $\Delta G_{\text{bilayer}}^\ddagger$. This conclusion is reinforced by the results in Fig. 3 because the relation between ΔG_{13}^\ddagger and ΔG_{15}^\ddagger can be described by a shared function despite the different amphiphile structures, the different lipid structure, and the opposite chiralities of the 13- and 15-residue channel-forming subunits. Specific amphiphile-channel interactions thus do not contribute to the changes in τ , which means that the amphiphiles will have little effect on $\Delta G_{\text{channel}}^\ddagger$.

The linear relation between ΔG_{13}^\ddagger and ΔG_{15}^\ddagger therefore reflects also a linear relation between $\Delta G_{13,\text{bilayer}}^\ddagger$ and $\Delta G_{15,\text{bilayer}}^\ddagger$.

The shared linear relation between $\Delta G_{13,\text{bilayer}}^\ddagger$ and $\Delta G_{15,\text{bilayer}}^\ddagger$ indicates that the effects of amphiphiles can be described by a single parameter that characterizes the changes in bilayer elastic properties. Because the same linear relation applies to compounds that cause positive [TX100 (12)] and negative [capsaicin (12), curcumin (31), and docosahexaenoic acid (25)] changes in intrinsic curvature, the curvature-dependent contributions to $\Delta G_{\text{bilayer}}^\ddagger$ are likely to be small. In this case, one can approximate $\Delta G_{\text{bilayer}}^\ddagger$ as

$$\Delta G_{\text{bilayer}} = H_B \cdot (l - d_0)^2, \quad [3]$$

where the bilayer elastic properties are summarized by a single phenomenological spring constant, H_B (e.g., ref. 4). [Our conclusion, that the curvature contributions to $\Delta G_{\text{bilayer}}^\ddagger$ are small, does not depend on the use of planar bilayers formed using decane. Using a fluorescence quench-based method (32) that employs hydrocarbon-free large unilamellar vesicles to determine amphiphile-induced changes in lipid bilayer properties, as sensed by gA channels, we find that TX100 and capsaicin have similar effects—and that all compounds tested have similar effects when assayed in the planar bilayer and the lipid vesicle system.]

The bilayer contribution to ΔG^\ddagger then is given by

$$\begin{aligned} \Delta G_{\text{bilayer}}^\ddagger &= H_B \cdot [(l + \delta - d_0)^2 - (l - d_0)^2] \\ &= H_B \cdot \delta \cdot [2 \cdot (l - d_0) + \delta], \end{aligned} \quad [4]$$

and, if the amphiphile effect on $\Delta G_{\text{bilayer}}^\ddagger$ can be described by changes in H_B :

$$\frac{\Delta \Delta G_{\text{bilayer},13}^\ddagger}{\Delta \Delta G_{\text{bilayer},15}^\ddagger} = \frac{\Delta H_B \cdot \delta \cdot [2 \cdot (l_{13} - d_0) + \delta]}{\Delta H_B \cdot \delta \cdot [2 \cdot (l_{15} - d_0) + \delta]} = \frac{2 \cdot (l_{13} - d_0) + \delta}{2 \cdot (l_{15} - d_0) + \delta}, \quad [5]$$

which is independent of ΔH_B . As a quantitative check of Eq. 5, we note that the hydrophobic length of the AgA(15) channel, l_{15} , is ~ 2.2 nm (16, 18, 33) and the channel length varies ~ 0.08 nm/residue (e.g., ref. 4); therefore $l_{13} \sim 1.88$ nm. Using these values and the estimates for $\Delta \Delta G_{13}^\ddagger / \Delta \Delta G_{15}^\ddagger$ from Fig. 3C, we find that $d_0 \sim 4.0$ nm for bilayers formed using either DPhPC or DOPC. For comparison, the bilayer thickness at an applied potential of 200 mV, as estimated from capacitance measurements (assuming a dielectric constant of 2), is ~ 4.2 nm for DPhPC/n decane (21) and between 3.7–4.3 nm for DOPC/n decane (24, 25). The agreement between the measured bilayer thickness and the prediction based on Eq. 5 is striking and supports the use of Eqs. 1–4 to describe the energetic coupling between gA channel function and lipid bilayer properties.

It is thus possible to evaluate the changes in H_B from the changes in τ , using the relation

$$\Delta H_B = k_B T \cdot \frac{\ln\{\tau/\tau_{\text{cntl}}\}}{\delta \cdot [2 \cdot (l - d_0) + \delta]}, \quad [6]$$

which is obtained from Eqs. 1 and 4. For $l - d_0 = 1.8$ nm and $\delta = 0.16$ nm, either 200 nM GsMTx4 or 3 μ M TX100 cause a 4 kJ/(mole \cdot nm²) decrease in H_B , which should be compared to H_B in unmodified phospholipid bilayers, ~ 56 kJ/(mole \cdot nm²) (4). Even relatively modest changes in H_B , thus, may cause measurable changes in channel (membrane protein) function.

The amphiphile-induced changes in H_B , as measured using gA channels formed by 15-residue subunits, are transferable in that they provide for quantitative predictions of changes in $\Delta G_{\text{bilayer}}^\ddagger$ —

and thus lifetime—for channels formed by 13-residue subunits in a bilayer of the same composition (and vice versa); cf. Fig. 3. They also are scalable, in that they provide for quantitative descriptions of the effects of amphiphiles on membrane protein function involving entirely different structural changes in plasma membranes. Fig. 4 shows the effects of five amphiphiles (TX100, reduced Triton X-100, β -octyl glucoside, Genapol X-100, and capsaicin) on the membrane potential for 50% inactivation (V_{in}) of voltage-dependent sodium channels in HEK293 cells, as function of changes in H_B measured using gA channels in DOPC/n-decane bilayers (based on refs. 12 and 27). Despite the structural differences—and that capsaicin promotes opposite curvature from the other molecules—the V_{in} vs. H_B relations vary little among the different amphiphiles.

Given the many different effects that amphiphilic compounds can have on the physical properties of lipid bilayers, it is perhaps surprising that the effects of structurally different amphiphiles on the bilayer elastic response can be characterized by changes in a single parameter, a phenomenological bilayer spring constant H_B . The finding represents an important conceptual simplification of the bilayer-mediated effects of amphiphiles and, moreover, provides a quantitative measure (a number) that can be used in further mechanistic studies. That the amphiphiles decrease H_B (and the energetic cost of deforming the bilayer) most likely reflects that water-soluble amphiphiles reversibly adsorb to lipid bilayers and thereby decrease the bilayer elastic moduli (14).

Though long recognized, the regulation of membrane protein function by membrane-active compounds has remained elusive because it has been difficult to obtain quantitative information about changes in the bilayer properties that are relevant for protein function. Nevertheless, studies on amphiphile regulation of membrane proteins often are done at concentrations that alter bilayer physical properties (see ref. 4 for a list of examples). The issue becomes particularly important in drug discovery and development—most orally available drugs are amphiphiles (34), and increasing drug lipophilicity has become a major and rising cause of nonspecific drug actions and attrition (10, 11). The gA channel-based approach provides the advantage of a direct readout of amphiphile-induced changes in bilayer properties, as experienced by an embedded protein. It provides a tool for quantitative and predictive explorations of the bilayer-mediated

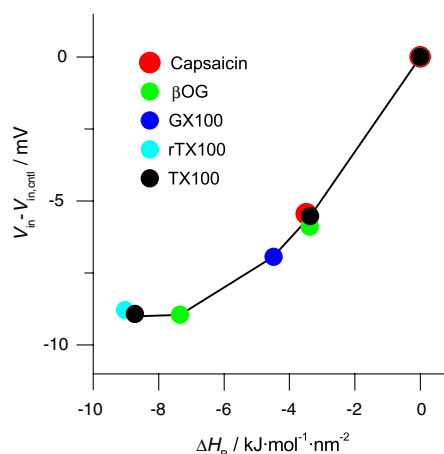


Fig. 4. Amphiphile-induced changes in inactivation of voltage-dependent sodium channels in HEK293 cells as a function of changes in H_B in DOPC/n-decane bilayers. Shift in membrane potential for 50% inactivation ($V_{\text{in}} - V_{\text{in,ctrl}}$) plotted vs. $\Delta H_B (=H_B - H_{B,\text{cntl}})$ in DOPC/n-decane bilayers. Amphiphiles: capsaicin, β -octyl puranoside (β OG), Genapol X-100 (GX100), reduced Triton X-100 (rTX100), and TX100. HEK293 cells were depolarized to +20 mV following 300-ms prepulses to potentials varying from -130 to $+50$ mV. Based on results from refs. 12 and 27.

regulation of membrane protein function by drug candidates and other amphiphiles.

Materials and Methods

Gramicidin analogues [des-Val¹-Gly²]gA⁻ and [Ala¹]gA were synthesized as described by ref. 20. Single-channel measurements using Triton X-100 were done at 25 °C in DPhPC or DOPC (Avanti Polar Lipids)/*n*-decane bilayers, separating 1.0 M NaCl, 10 mM Hepes, pH 7 solutions using the bilayer punch method, as described previously (4). The applied potential was ±200 mV. The

gA analogues and amphiphiles were added to both sides of the bilayer. Survivor plots of channel lifetimes were fitted by a single exponential distribution, $N(t)/N(0) = \exp\{-t/\tau\}$, where $N(t)$ is the number of channels with duration longer than time t , and τ is the average lifetime (12, 22, 24–28).

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