

## Interfacial Polar Interactions Affect Gramicidin Channel Kinetics

Tatiana K. Rostovtseva,\* Horia I. Petrache,<sup>†</sup> Namdar Kazemi,\* Elnaz Hassanzadeh,\* and Sergey M. Bezrukov\*

\*Laboratory of Physical and Structural Biology, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892; and <sup>†</sup>Department of Physics, Indiana University-Purdue University Indianapolis, Indianapolis, Indiana 46202

**ABSTRACT** Critical to biological processes such as secretion and transport, protein-lipid interactions within the membrane and at the membrane-water interface still raise many questions. Here we examine the role of lipid headgroups in these interactions by using gramicidin A (gA) channels in planar bilayers as a probe. We show that although headgroup demethylation from phosphatidylcholine (DOPC) to phosphatidylethanolamine decreases the lifetime of gA channels by an order of magnitude in accordance with the currently accepted hydrophobic mismatch mechanism, our findings with diether-DOPC suggest the importance of the headgroup-peptide interactions. According to our x-ray diffraction measurements, this lipid has the same hydrophobic thickness as DOPC but increases gA lifetime by a factor of 2. Thus we demonstrate that peptide-headgroup interactions may dominate over the effect of hydrophobic mismatch in regulating protein function.

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Address reprint requests and inquiries to Tatiana K. Rostovtseva, Tel.: 301-402-4702; Fax: 301-496-2172; E-mail: rostovtt@mail.nih.gov.

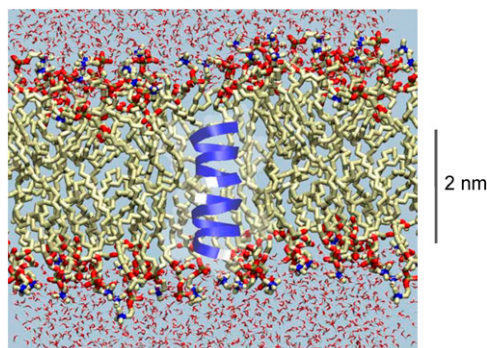
Residing within the inner oily part of the membrane, trans-membrane proteins are significantly affected by nonspecific, hydrophobic interactions (1–4). To quantify tractable energetic contributions of these interactions within an ingenious physical model, the concept of hydrophobic mismatching was introduced. In essence, in this model, any mismatch in hydrophobic dimensions between the protein and the lipid incurs an energetic penalty causing lipid and protein deformations or structural adaptations (4,5). Many studies, including this one, support this insightful model. In this Letter, we use the benchmark antibiotic gramicidin A (gA) to show that the polar part (the “other part”) of the lipid bilayer claims its own role in lipid-channel interactions. Here we compare the dissociation rate of single gA channels in “solvent-free” planar bilayers made of dioleoyl-phosphatidylcholine (DOPC), dioleoyl-phosphatidylethanolamine (DOPE), and diether-DOPC (DEPC) lipids (or mixtures), and in addition, we evaluate the effect of monovalent salt concentration.

The gA channel is formed when two gA monomers residing in opposite monolayers bind at the membrane center forming ion-conductive dimers (6). Given that the channel length,  $\sim 2.2$  nm (Trp-Trp distance) (7), is less than the thickness of a typical lipid bilayer,  $\sim 4$  nm (8) (Fig. 1), channel formation involves compression and bending of lipid monolayers, as captured by the hydrophobic mismatch models and supported by both x-ray (9) and <sup>2</sup>H NMR (10) studies. Because of this, the kinetics of gA channel formation and dissociation are sensitive to bilayer thickness and consequently to the local bilayer environment (7,11). The equilibrium constant of channel dimerization and channel lifetime can be correlated not only with the bilayer thickness, but with elastic moduli and curvature stresses (3,11,12).

However, lipid bilayers are more than just hydrophobic slabs of materials. Although difficult to analyze because of

its complexity, the polar part of lipid bilayers, involving lipid headgroups, carbonyl groups, and interfacial water, is neither negligible nor inert. In terms of size, the x-ray measurements mentioned above have shown that the polar thickness of membranes accounts for 30–40% of the total membrane thickness (8), depending on the length of acyl chains (13).

How to distinguish between the specific and nonspecific role of a lipid polar part? For example, demethylation of PC headgroups to PE leads to increased hydrocarbon thickness and decreased lifetime of gA channels (Fig. 2). Is it chemistry or physics that affects the gA? To quantify the effect of demethylation, note that the cross-sectional area per lipid molecule changes from  $0.72$  nm<sup>2</sup> in lamellar DOPC (8) to  $0.64$  nm<sup>2</sup> in nonlamellar DOPE (14). A much closer packing of PE lipids results in increased bilayer thickness. Measured



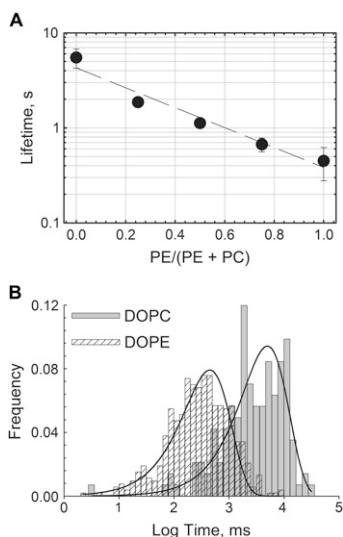
**FIGURE 1** Snapshot of a molecular dynamics simulation of a gramicidin dimer in a DOPC bilayer emphasizes the spatial extent of the membrane polar regions.

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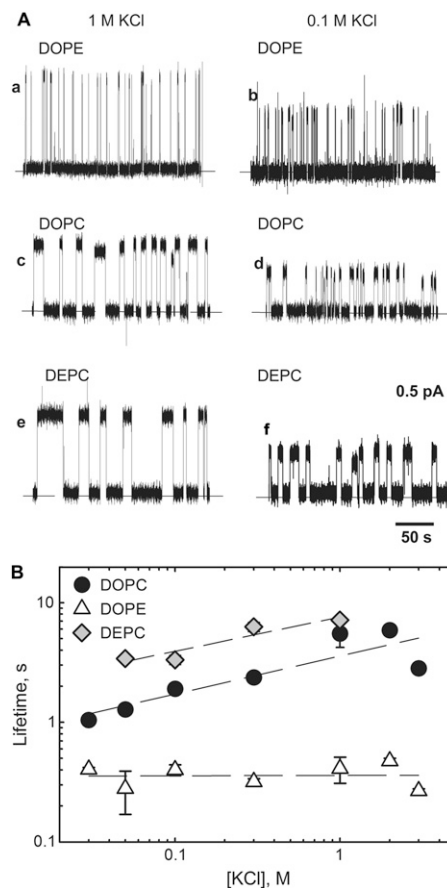
by x-ray diffraction, the hydrophobic thickness of DOPE bilayers is 3.04 nm (14) and that of DOPC is 2.73 nm (8). Thus, the hydrophobic thickness of DOPE bilayers is larger than that of DOPC by 0.3 nm, leading to a significant increase in the mismatch between the length of the gramicidin dimer and bilayer thickness. Gramicidin lifetime appears to be a good measure of the hydrophobic changes. As the bilayer becomes thicker with the addition of PE headgroups, gA lifetime decreases (Fig. 2)—a behavior well captured in the hydrophobic matching models.

Here we consider three different ways to modify the physical properties of the membrane polar part: i), demethylation of PC to PE, ii), modification of acyl link (DEPC), and iii), addition of salt at the lipid-water interface. Each of these modifications alters physical interactions at the lipid-water interface. We use lipids with the same oleoyl acyl chains but with different polar parts. DEPC has a simple C-O-C ether segment in place of the COO-C carboxyl of the first two lipids.

Representative current traces of gA channels in the membranes made from different lipids in 1.0 and 0.1 M KCl solutions are shown in Fig. 3 A. The increase of gA lifetime with salt concentration (Fig. 3 B) is similarly substantial for the two PC lipids, whereas for DOPE the lifetime is practically unchanged. The increase of the lifetime measured for PC lipids is not due to changes in membrane thickness simply because the thickness is practically unaffected by K salts (15). Moreover, high ionic strengths have a dehydration effect on PC headgroups, thus altering headgroup interactions only to increase membrane thickness, although these changes are small (15). Therefore, in the standard hydrophobic mismatch model, one would expect a decrease of gA lifetime with addition of salt, contrary to experimental results. Note that gA lifetime in DOPE is not affected by salt



**FIGURE 2** gA channel lifetime may reflect hydrophobic changes. (A) gA lifetime in PC decreases with addition of PE. (B) The lifetime histograms in DOPC and DOPE membranes. The medium consisted of 1 M KCl, 5 mM HEPES, pH 7.4.

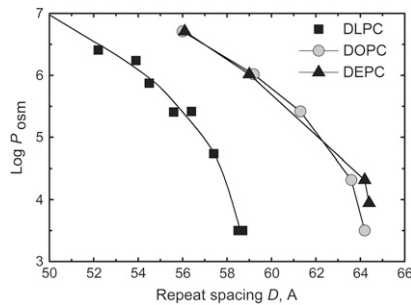


**FIGURE 3** Channel lifetime increases when DOPC is substituted by DEPC and depends on salt concentration for these lipids but not for DOPE. (A) Current traces for bilayers formed from DOPE (a and b), DOPC (c and d), and DEPC (e and f) in 1 M (a, c, and e) and 0.1 M (b, d, and f) KCl solutions. The applied voltage was 50 mV. Current records were filtered using averaging time of 30 ms. Dashed lines indicate zero current level. (B) Summary of the statistical analysis of all experiments (21).

because PE headgroups have a much smaller hydration shell than the PCs and are harder to dehydrate.

One cannot easily distinguish between a hydrophobic and a polar effect by comparing DOPC and DOPE, because both effects are present. The third lipid that we considered, DEPC, helps make this distinction. The important observation is that the hydrophobic thickness of DEPC obtained by direct x-ray scattering measurements at 25°C is essentially the same as of DOPC (Fig. 4), whereas gA lifetime doubled (Fig. 3). Lipid carbonyls affect gA channel stability but in the opposite direction of what was anticipated (16), where hydrogen bonds between PC carbonyl and gA indole were proposed to stabilize gA-conducting dimer.

Similar stabilizing effects of high salt in both ether and ester PC suggest the importance of Trp residues and lipid nitrogen-cation interactions, if we assume that Trp residues play a dominant role in gA-lipid interaction (17). Thus, we conclude that salt-modified gA interactions with lipid head-



**FIGURE 4** Hydrophobic thickness of DEPC membrane is practically the same as DOPC. Osmotic pressure for DOPC, and DEPC multilamellar samples versus the repeat spacing at 25°C. For comparison, data for a thinner DLPC membrane are presented.

group alter gA lifetime and that this mechanism dominates over the effect of hydrophobic mismatch. In contrast to the previous studies (16,18), our results suggest that hydrogen bonding between gA indoles and lipid carbonyls does not play a significant role in gA dimer channel stabilization. Our results are in a good agreement with Providence et al. (19), who concluded that ester carbonyl groups in DOPC do not interact with the indole moieties in gA channels.

The previously reported increase of gA channel lifetime with salt concentration in glycerylmonooleate membranes was interpreted in the framework of the occupancy hypothesis (20), wherein the channel stability increases with ion occupancy of the channel. Our results with DOPE contradict this prediction and demonstrate that the lifetime is more influenced by gA interaction with lipid headgroups and by hydrophobic mismatch.

Using simple arguments (12), we estimate the standard free energy difference of gA channel dimer in DOPC and DOPE membranes to be  $\sim 2.5$  kT per channel or  $\sim 1.25$  kcal/mol. Interestingly, Scarlata and Gruner (18) calculated the enthalpy involved in the loss of hydrogen bonds in PE between Trp residues and lipid polar region as  $\sim 2$  kcal/mol. These authors speculated that this loss may be related to an increase in the distance between the gramicidin indoles and PE headgroups due to the smaller size of the PE headgroup, or due to the decrease of PE-gA association. According to our data, most likely the latter is taking place.

To conclude, our experiments demonstrate that even in the simple case of a gA channel, its regulation involves both nonspecific (hydrophobic mismatch) and specific (headgroup-peptide) interactions, thus highlighting the importance of the latter in the functioning of membrane proteins.

## SUPPLEMENTARY MATERIAL

To view all of the supplemental files associated with this article, visit [www.biophysj.org](http://www.biophysj.org).

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- gA channel lifetime does not depend on the magnitude of the applied voltage. See Fig. S2A in the Supplementary Material.