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Role of phosphatidylethanolamine lipids in the stabilization of protein–lipid contacts

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Abstract

We have investigated the effect of lipids with phosphatidylethanolamine (PE) head groups on the stabilization of contacts between the tryptophan side chains of gramicidin and the lipid head groups. We initially developed two fluorescence methods that can be correlated to the spontaneous curvature of DOPC/DOPE and DOPC/DOPEme. One is based on bilayer structure and measures the rotational motion of a probe located close to the membrane surface relative to a more deeply-buried probe. The second is based on surface hydration/polarity and measures the emission energy of a polarity-sensitive probe located on the membrane surface. We used these methods to estimate the pseudo-curvature (i.e., curvature obtained by fluorescence measurements) of lipids with dimyristyl chains, and their pressure and temperature dependence. We then investigated the stability of gramicidin tryptophan–lipid contacts in DMPC/DMPE as a function of temperature and pressure. Stability was assessed by tryptophan rotational motion as determined by fluorescence anisotropy, since rotational motion is limited when the indoles are hydrogen bonded to the lipid head groups. The results suggest that the presence of PE lipids destabilizes these contacts due to either their smaller size relative to PC head groups, or their tendency to self-interact. Fluorescence quenching studies support these results. © 1997 Elsevier Science B.V.

Keywords: Phosphatidylethanolamine; Gramicidin; Curvature; Hydrogen bonds; Fluorescence; High pressure

1. Introduction

The physiological role of lipids with phosphatidylethanolamine headgroups (PE) is unclear, even though these lipids are prevalent in biological membranes. The presence of PE lipids has been shown to have varying effects on biochemical processes including increasing the association of peripheral proteins, altering the properties of transport proteins, increasing the propensity for membrane fusion and altering the infectivity of viruses [1–3]. Here, we investigate the possibility that PE lipids may alter the contact proteins make with the lipid head groups using the peptide gramicidin as a model system. Gramicidin is chosen because it can be reconstituted into bilayers to give a defined N-terminal to N-terminal helical dimer whose conformation can be verified

Abbreviations: DOPE, DMPE, dioleoyl- and dimyristoyl-phosphatidylethanolamine; DOPC, DMPC, dioleoyl- and dimyristoyl-phosphatidylcholine; DOPEme, dioleoyl-phosphatidylmethylethanolamine; Laurodan, 6-dodecanyl-2-(dimethylamino)-naphthalene; GR, gramicidin D; 2AS, 12AS, 2- and 12- anthroyloxystearic acid; dipyrenylPC, di(6-pyrenylstearyl)-phosphatidylcholine

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by CD [4]. In this form the four peptide tryptophans, which can be probed by fluorescence emission, are near the membrane surface. More importantly, we used gramicidin because the indole protons have been shown, by fluorescence and NMR studies, to hydrogen bond to the lipid head groups and stabilize this conformation [5–7]. Previous fluorescence studies indicate that these hydrogen bonds can be disrupted by inducing hydrophobic mismatch between the protein and the lipid. Breakage of the hydrogen bonds is followed spectroscopically by the increase in rotational motion of the indole group [5].

While phosphatidylcholine (PC) lipids form planar bilayers, PE lipids have a strong tendency to curve into hexagonal phases (for review see [8]). When incorporated into bilayers, PE lipids are also known to destabilize bilayers by increasing the spontaneous curvature of the monolayer leaflets which make up the membrane [9,10]. This behavior is contrasted with PC lipids, which decrease the spontaneous curvature and stabilize bilayers. These two classes of lipids differ only by the degree of methylation of the quaternary nitrogen in the headgroup: The three methyl groups of PC are each replaced with hydrogens in PE. This substitution both reduces the headgroup molecular volume, which is a relatively small change, and increases the propensity to hydrogen bond, which is thought to be a major change, affecting both the polarity and packing of the membrane surface. There is only a poor understanding of the details whereby volume and hydrogen bonding changes translate into both altered interactions with proteins or changes in the monolayer spontaneous curvature. Hence, we investigated the effects of substituting PE for PC upon the correlation between monolayer spontaneous curvature and effects of gramicidin.

Gramicidin is most soluble in lipids with shorter hydrocarbon chains (less than 16 CH_2; see [11]) but most of the characterization of the spontaneous curvature of PE lipids has been done using lipids with longer, and unsaturated, hydrocarbon chains. Therefore, it was first necessary to develop methods which correlate spontaneous curvature in long-chain, unsaturated lipids to fluorescent effects with shorter-chained, saturated lipids. These correlations were then used when monitoring the stability of gramicidin–lipid hydrogen bonds as a function of temperature and pressure. Our results indicate that Trp–lipid interactions are destabilized by PE lipids.

2. Materials and methods

2.1. Materials

Lipids were obtained from Avanti Polar Lipids (Alabaster, AL). Fluorescent probes were obtained from Molecular Probes, (Eugene, OR). Gramicidin was from Calbiochem. DipyrenylPC was a generous gift from Dr. Massimo Sassaroli (Mt. Sinai School of Medicine, New York). Unless otherwise specified, all samples were buffered in 50 mM HEPES, pH 7.0, 0. 16M NaCl.

All studies were done at temperatures where the lipids were in fluid phase to avoid the possibility of phase separation and domain formation. Multilamellar vesicles were used for experiments involving lipids with oleyl chains since previous studies have used lipid in this form (e.g., [9]). These vesicles were prepared by mixing the lipids in chloroform, drying to a thin layer under vacuum using a rotary evaporator, and hydrating the lipid in buffer by brief sonication in a bath sonicator. Samples were made in 2 mM lipid stocks and stored under nitrogen at 5°C. In the fluorescence studies, the lipids were diluted 10–20 fold, and the probes added from concentrated stock solutions in ethanol to give a 0.5 to 1% labeling ratio. To aid the partitioning of the detergent probes into the multilayers, the lipids were then sonicated briefly in a bath sonicator. Studies using anthroyloxy fatty acid probes were done at pH 5 to avoid being close to the pH of stearic acid (pH 7) since changes in protonation may alter the position of the probe in the membrane [12]. We note that similar, but less reliable, data were obtained at pH 7.

For the studies involving lipids with myristoyl chains both multilamellar and unilamellar vesicles were used. Gramicidin studies used only unilamellar vesicles because we wanted to study gramicidin in its N-terminal to N-terminal dimer form rather than its intertwined helical dimer, and the simplest way to achieve this is by reconstituting the protein by cosonication with the lipids, which produces unilamellar vesicles. Unilamellelar vesicles were prepared by sonicating the hydrated multilayers in a cup horn

sonicator (Heat Systems, Farmingdale, NY) for 20′ at full power. Bilayers were separated from multilayers by sedimentation at 20,000 g for 30′. For the studies using the diprenylPC probes, the probes were mixed with the lipids at a 1 mol% ratio in chloroform and the chloroform evaporated by a stream of nitrogen and then under vacuum. For the gramicidin studies, the peptide was mixed with lipid in either chloroform or ethanol (both gave identical results) before drying at a peptide:lipid ratio of 1:50. CD spectra taken of the final bilayers were consistent with the N-terminal to N-terminal conformation of the peptide [4].

2.2. Methods

Fluorescence data were taken on an I.S.S. K-2 phase-modulation spectrofluorometer (I.S.S., Champaign, IL). Laurodan spectra were measured using an excitation wavelength of 340 nm and scanning the emission from 380 to 580 nm. Laurodan data are reported in terms of the center of spectral mass. The data are an average of a minimum of three sets of individual samples. The anisotropy measurements of the AS probes were made using excitation and emission wavelengths of 381 and 460 nm, respectively. For gramicidin studies, an excitation wavelength of 292 nm and an emission wavelength of 340 nm were used. Corrections for background scattering were made using unlabeled lipids. The anisotropy of each sample was measured 5–15 times using 3–5 independent sets of samples. Anisotropy measurements of gramicidin were made by matching the scattering intensity of the proteoliposomes with unlabeled sample at 500 nm, and correcting for background.

The rotational correlation time, R, is determined by measuring of the anisotropy and lifetime, τ, through the Perrin equation:

$$\frac{A_0}{A-1} = RT\frac{\tau}{\eta V} \quad (1)$$

where A_0 is the anisotropy in the absence of rotational motion and has been found to be 0.312 for 2AS and 12AS at $\lambda_{exc} = 381$ and $\lambda_{em} = 460$ nm, A is the anisotropy, T is the absolute temperature, η is the viscosity, τ is the fluorescence lifetime and V is the rotational volume assuming a sphere.

3. Results

Initially, we developed two fluorescence methods that will enable us to correlate spectroscopic changes to spontaneous curvature in PE lipids as determined by X-ray diffraction (Section 3.1). These correlations were then used to measure the pseudo-curvature of lipids with dimyristoyl chains (Section 3.2), where the word 'pseudo-curvature' is used to remind the reader that the spontaneous curvature has not been directly measured for the shorter chain lipids. The pseudo-curvature is then related to changes as a function of temperature and pressure (Section 3.3), and in the presence of protein (Section 3.4). Finally, these results were then used to determine the stability of gramicidin–lipid contacts in the presence of PE lipids (Section 3.5).

3.1. Part I-Fluorescence assessment of DOPC–DOPE and DOPC–DOPEme spontaneous curvature

3.1.1. Fluorescence anisotropy

We have correlated monolayer spontaneous curvature to fluorescence by two methods: one that is closely related to relative changes across the depth of the membrane with increasing PE content and another that involves changes in the hydration of the membrane surface with PE content. The first method is based on the comparison of the fluorescence anisotropy of two stearic acid probes. In one, 2-AS, the fluorescent anthroyloxy side chain is close to the head group region of the lipid and in the second, 12AS, the side chain is buried in the hydrophobic core [13]. The fluorescence anisotropy, which describes the amount of rotation motion the probe undergoes during the excited state, can be related to the cosine squared of the average precession angle swept out during the rotation (see Eq. (2) below). We reasoned that in a cylindrically-shaped lipid, the presence of hexagonal-phase forming lipids would increase the packing of the surface relative to the interior. Thus, the anisotropy of the probe located closer to the surface, 2AS, should increase with increasing PE or PEme concentration as compared to 12AS. It is important to note that the anisotropy data obtained here is related to the rotational volume of the probes and, as described below, directly related

to structural changes brought about by the incorporation of PE into the PC membranes. Any changes in hydration or polarity are only indirect. This behavior contrasts the second method used in this paper.

We measured the anisotropy of DOPC multilayers doped with 1 mol% 2AS or 12AS with increasing PE content. In accord with previous studies, we found that under our conditions (i.e., 0. 16M NaCl, pH 5, 22°C) the vesicles remained in the bilayer phase during their time of use (1–3 days) up to about 80 mol% PE where bilayers could not be formed. Our data show that the anisotropy of the 2AS probe increased with increasing PE and PEme content, while the anisotropy of 12AS in either of the two lipid systems was found to remain relatively constant. Data obtained for the relative quantum yields of the probes indicated that self-quenching of the probes does not occur at any PE concentration. Self-quenching of the probes, especially at low PE content, would be indicative of unequal partitioning of the probes. This result, along with the fact that the data were always collected in fluid phase membranes composed of lipids having identical hydrocarbon chains, argues against the observed changes reflecting local effects. Note also that time scale of the fluorescence lifetime of the probes is much greater than the diffusion rate of fatty acids in membranes.

In Fig. 1 we show the anisotropy data, normalized to 1.00 at 100% DOPC, for PC/PE and for PC/PEme mixtures. These data indicate that the changes in membrane structure brought about by increasing amounts of hexagonal phase forming lipids do not cause significant changes in lipid packing in the hydrophobic core, only in the head group region.

The anisotropy can be related to the precession angle (θ) inscribed the rotating fluorophore during its lifetime (the lifetime does not change significantly with PE incorporation) by:

$$\cos^2(\theta) = \frac{\left[1 + 2\left(\frac{A_0}{A}\right)\right]}{3} \qquad (2)$$

This precession angle can be regarded as a volume element from which we can define an angular ratio, $\boldsymbol{B}$, which is related to the volume elements in the two regions of the membrane where the probes are located [14]: $\boldsymbol{B} = (\theta_{2AS}/\theta_{12AS})$. We find that the angular ratio, $\boldsymbol{B}$, changes systematically with increasing amount of either PE or PEme. Also, the change in anisotropy and $\boldsymbol{B}$ with concentration is similar for PE and PEme when these data are plotted in terms of mol% of PE or PEme (Fig. 2a) or monolayer spontaneous curvature (Fig. 2b), where the data given in Keller et al. (1993) [15], have been used to translate from mole ratios of the lipids to curvature.

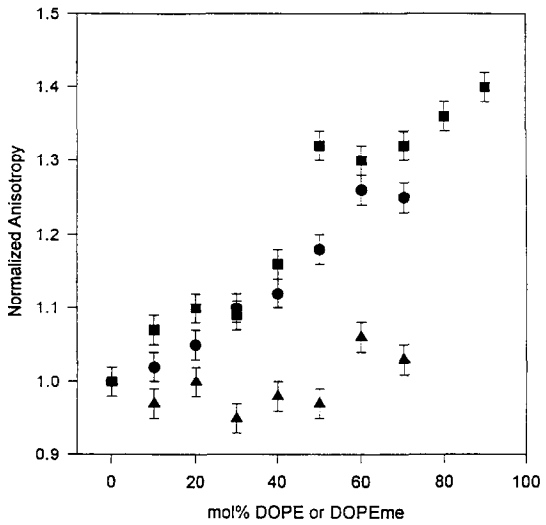

Fig. 1. Normalized anisotropy of 2AS in DOPC with increasing amounts of DOPE (●) or DOPEme (■). (▲) are data for 12AS in DOPC/DOPE mixtures.

3.1.2. Fluorescence energy shifts

In conjunction with the anisotropy studies, a second method was tested. Fully hydrated PE lamellae have less water between the lamellae than their PC counterparts, suggesting that the PE surface has a lower affinity and/or capacity for water. With this in mind, we studied the effect of increasing amounts of PE or PEme into DOPC membranes on the center of spectral mass (which is the weighted emission energy) of the fluorescence probe, Laurodan. The emission energy of the fluorescent head group of Laurodan has been shown to be highly sensitive to changes in the surrounding dielectric constant [16,17]. Increasing the amount of either PE or PEme shifts the distribution towards the higher energies (data not shown). As was the case for the angular ratio, the changes in the center of mass with mol% PE and

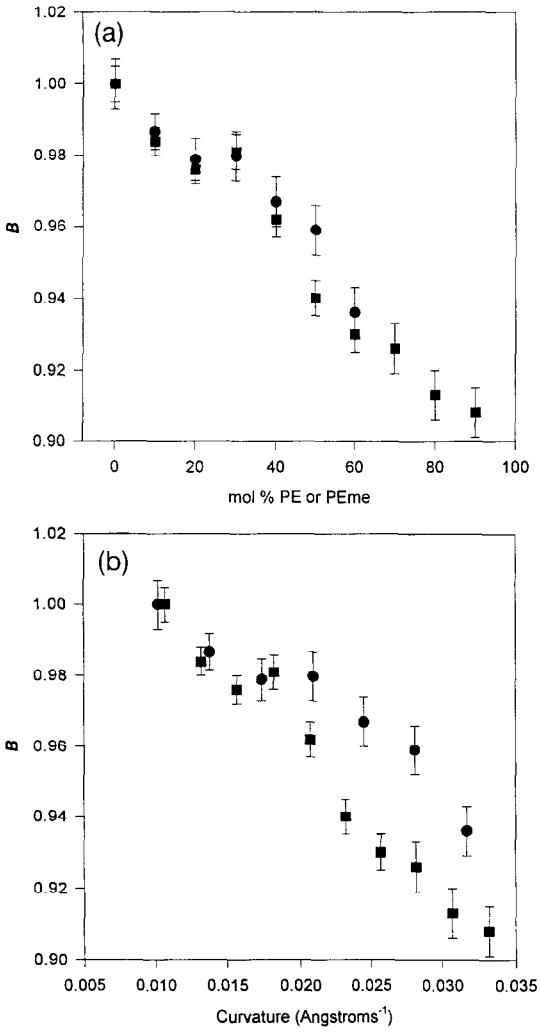

Fig. 2. (a) The angular ratio ($\boldsymbol{B}$) as a function of DOPE (●) or DOPEme (■) in DOPC. $\boldsymbol{B}$ values were obtained from the ratio of the precession angles, θ, determined by anisotropy measurements of 2AS and 12AS (see text); (b) $\boldsymbol{B}$ values from Fig. 2a plotted as a function of spontaneous curvature or C as calculated by $C_{mix} = xC_{DOPE} + (1-x)C_{DOPC}$ where x is the mol fraction of DOPE; see Keller et al. (1993) [15].

PEme, and with spontaneous monolayer curvature, are similar in both lipid systems (Fig. 3).

3.2. Part II-Extension of fluorescence correlation to DMPC / DMPE lipids

Although the bilayer to hexagonal phase transition of DMPE has been characterized, a direct spontaneous curvature measurement of DMPC/PE mixtures, similar to that done in Keller et al. [15], has not been reported. Therefore, we measured the response of the fluorescence probes used in Part I in DMPC membranes as increasing amounts of DMPE was added. Curves fit to the data of Fig. 2a and Fig. 3, were used to correlate the angular ratio and the Laurodan spectral center of mass, respectively, to a spontaneous curvature. Since there is only an incomplete understanding of the molecular mechanisms leading spontaneous curvature or the fluorescence responses, there is no guarantee that the correlation yields the true myristoyl lipid spontaneous curvatures; therefore, we shall call curvatures determined via the fluorescence correlations of Fig. 2b and Fig. 3 'pseudo-curvatures'. The resulting plots are shown in Fig. 4. We note that the measurements of the lipids with myristoyl chains were performed at 30°C where the lipids are in the fluid phase whereas those for the lipids with dioleoyl chains were done at 22°C. This temperature difference would affect the relationship between the two types of lipids if the emission energy of Laurodan changed significantly in this temperature range. While there is a small difference in emission energy at these temperatures in both water and ethanol, it is small compared to the changes observed over this same temperature range in DMPC.

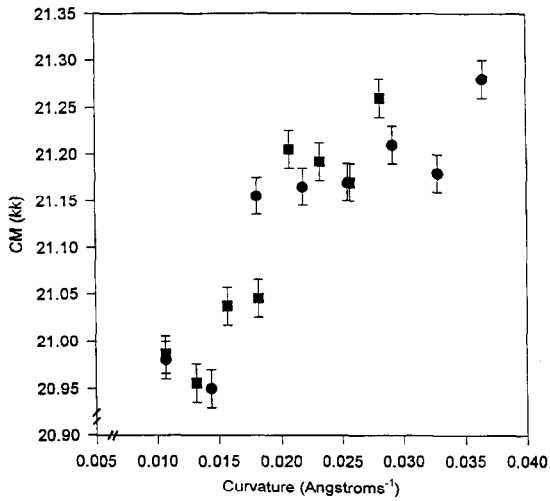

Fig. 3. Change in the emission energy of Laurodan, expressed as the center of spectral mass (CM) in kilokaisers (1 kk = 1000 cm^{-1}) with curvature in DOPC–DOPE (●) and DOPC–DOPEme (■) membranes.

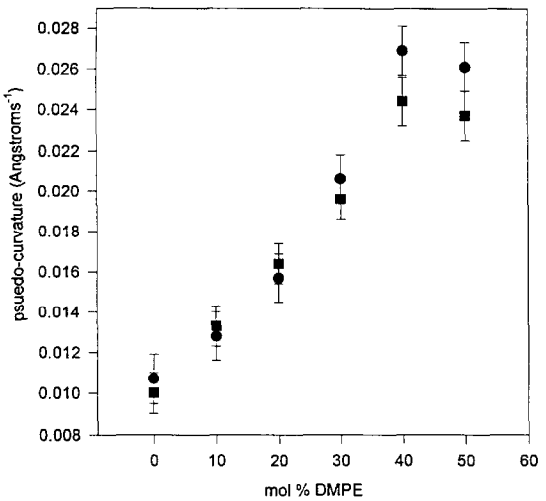

Fig. 4. Change in pseudo-curvature of DMPC with increasing DMPE as calculated from Laurodan emission (●) or the angular ratio, ***B*** (■).

3.3. Part III-Changes in pseudo-curvature with temperature and pressure

As described below, the strength of gramicidin–lipid head group interactions will be assessed by studying their stability as a function of temperature and pressure. Therefore, we characterized the temperature and pressure dependence of the pseudo-curvature of DMPC/PE mixtures. The temperature and pressure dependence of DOPC/PE mixtures has been previously studied by NMR and diffraction [15,18–21]. Raising the temperature increases the curvature of lipid monolayers. In an excess-water reversed hexagonal phase this is manifest as an increase cross-sectional areas of the ends of the hydrocarbon chains relative to the head groups. Pressure has the opposite effect of lowering the spontaneous curvature.

3.3.1. Characterization of changes of the gel–liquid crystal phase transition with increasing PE content

In the gramicidin studies we will monitor the protein–DMPC/PE interactions through the gel to liquid crystal phase transition. We first verified that the presence of PE lipids does not significantly change the transition temperature and pressure. For these experiments, we used a fluorescent PC probe whose fluorescent groups are embedded in the hydrocarbon region and are sensitive to the gel to liquid crystalline phase transition, e.g., [22]. In the gel phase the rotational motion of the two pyrenyl side chains are limited and this limited motion stabilizes pyrene excimers, or excited state dimers. The excimer and monomer emission spectra are quite distinct and easily resolvable by fluorescence. Upon entering the fluid phase the rotational motion of the probe increase which decreases the excimer population. By monitoring the monomer/excimer ratio we found that the incorporation of either 25 and 50 mol% DMPE raises the transition temperature approximately 2 to 4° respectively; and the pressure-induced transition pressure by approximately 50 and 100 b at 30°C (data not shown).

3.3.2. Temperature studies

We then determined the temperature dependence of 2AS and 12AS embedded in DMPC multilayers with 0, 25 and 50% DMPE. Both probes showed a smooth decrease in anisotropy and lifetime with temperature. From these data the angular ratio, ***B***, and the pseudo-curvature were calculated. The results, shown in Fig. 5, indicate that the effect of PE is not only to increase the membrane pseudo-curvature but also to shift the gel to liquid crystal phase transition to higher transition temperatures as seen by the shifts in the midpoints of the curves. Monitor-

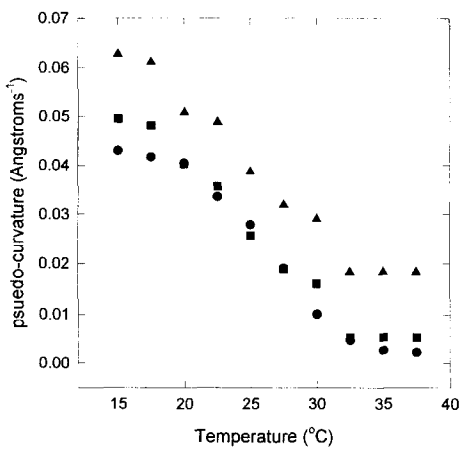

Fig. 5. Temperature dependence of the pseudo-curvature, as determined from ***B***, for 100% DMPC (●), DMPC–DMPE 75:25 (■) and DMPC–DMPE 50:50 (▲). The temperature dependence from Laurodan emission (not shown) gave similar results. Errors ranged from ±0.009 to ±0.0013.

ing the temperature dependence of pseudo-curvature using Laurodan produced similar behavior (data not shown).

3.3.3. Pressure studies

We studied the change in Laurodan emission in DMPC/PE mixtures as a function of pressure. Data were taken at 40°C. In the samples containing PE, the onset of the liquid crystal to gel phase transition occurs at increasingly lower pressures making it difficult to isolate changes in curvature in the fluid phase. We followed the emission energy of Laurodan embedded in DOPC multilayers in order to better define the changes in pseudo-curvature induced by pressure. We find that while the incorporation of PE lipids shifts the spectra of Laurodan to higher energies at atmospheric pressure (Fig. 2), pressure reverses this shift bringing the emission energy closer to that seen for Laurodan in pure DOPC; in polar solvents, the emission of the fluorescence headgroup of Laurodan will shift to lower energies with pressure due to increased interaction with the polar solvent as the volume decreases [23]. If we compare the shift in emission with PE content to the shift in emission with pressure, then we can estimate the pressure dependence of DOPC/PE pseudo-curvature. These curves, displayed in Fig. 6, show that as the membranes are compressed, pseudo-curvature decreases. This is qualitatively similar to the pressure dependence of monolayer spontaneous curvature observed in previous X-ray diffraction studies (see discussion).

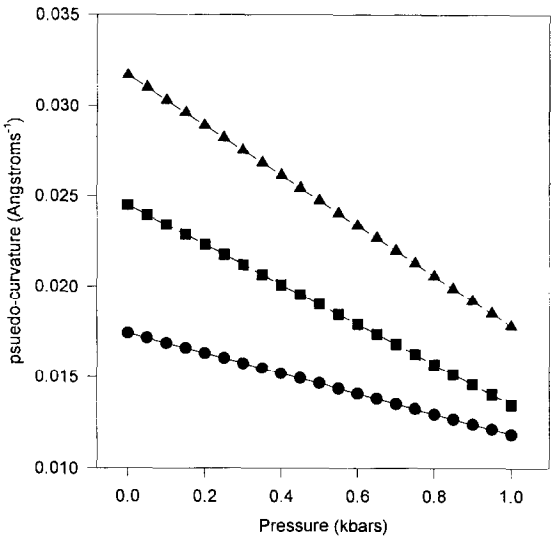

Fig. 6. Pressure dependence of the pseudo-curvature, as determined from ***B***, for 100% DMPC (●), DMPC–DMPE 75:25 (■) and DMPC–DMPE 50:50 (▲). Errors ranged from ±0.010 to ±0.0012.

3.4. Part IV-Characterization of the pseudo-curvature of bilayers containing gramicidin

The N-terminal to N-terminal form of gramicidin has been reported to have an 'inverse conical shape' due to the presence of the bulky tryptophan side chains close to the surface of the membrane. Thus, incorporation of gramicidin may reduce the curvature of the DMPC/PE membranes. To determine whether the presence of 20 mol% gramicidin (where we will be conducting the intrinsic fluorescence studies) alters membrane pseudo-curvature, we measured the dependence of 2AS and 12AS anisotropy and Laurodan emission at 0, 25 and 50 mol% DMPE in DMPC bilayers. We find that the incorporation of gramicidin reduces the pseudo-curvature of DMPC/PE membranes. The presence of 20% gramicidin reduces the pseudo-curvature of the 25% DMPE sample almost to that of the 0% DMPE samples. This behavior is consistent with the proposed effective shape of the gramicidin dimer.

3.5. Part V-Gramicidin studies

We studied the rotational properties of the tryptophan side chains of gramicidin in the N- to N-terminal dimer form where the indole protons can hydrogen bond to the lipids [5]. Previously, we have found that applying pressure to gramicidin in DMPC bilayers will disrupt these interactions since it will increase the length of the lipid due to straightening of the hydrocarbon chains without a concomitant increase in peptide length. When hydrogen bonds are present, the rotational motion of the indoles is limited. An increase in rotational motion is interpreted to correspond to disruption of these interactions.

In Fig. 7 we show the change in anisotropy of gramicidin tryptophans in DMPC/PE mixture as a function of pressure. We first note that at atmospheric pressure, the anisotropy of the Trp side chains decrease with increasing PE content corre-

sponding to an increase in rotational freedom of the indoles (the lifetime only changed 50 ps in this pressure range). In the absence of PE, the anisotropy first increased, and then decreased with pressure becoming close to the values seen in the presence of PE. This decrease in anisotropy is interpreted as being due to the disruption of indole–lipid hydrogen bonds. Incorporation of PE lipids eliminates this decrease at higher pressures and instead causes the anisotropy to increase under pressure. We note that the anisotropy of probes that cannot donate a hydrogen for hydrogen bonding to lipid carbonyls also increase under pressure [5] and that in general, the application of pressure is expected to reduced the rotational freedom of fluorophores.

Destabilization of tryptophan–lipid interactions by PE lipids was also studied as a function of temperature. In pure PC membranes, the tryptophan–lipid contacts are unchanged throughout the gel phase, as indicated by a constant rotational motion. But upon reaching the fluid phase, the rotational motion decreases due to a disruption of interactions between the indoles and the lipid head groups and to ring stacking interactions that have been found to occur between the Trps 9 and 15. The results, shown in Fig. 8, are consistent with the idea that lipid–peptide interactions are reduced in the presence of PE lipids.

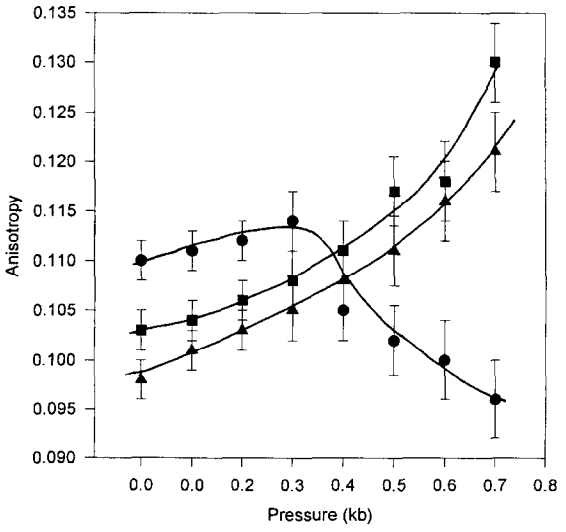

Fig. 7. Change in the anisotropy of gramicidin as a function of pressure in 100% DMPC (●), DMPC–DMPE 75:25 (■) and DMPC–DMPE 50:50 (▲). Lines are drawn to guide the eye.

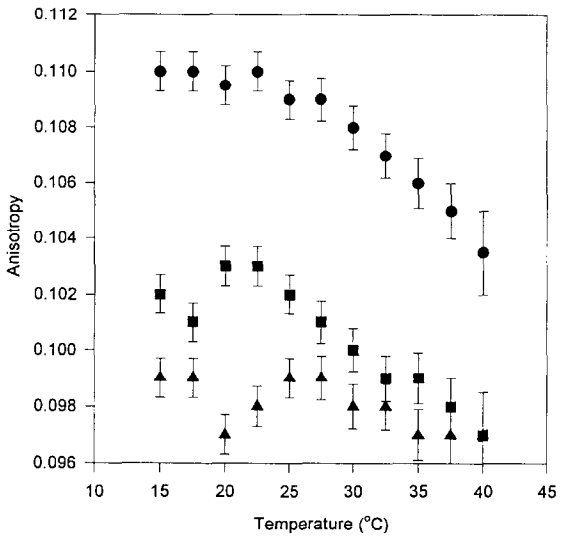

Fig. 8. Change in the anisotropy of gramicidin as a function of temperature in 100% DMPC (●), DMPC–DMPE 75:25 (■) and DMPC–DMPE 50:50 (▲).

If Trp–lipid contacts are destabilized by PE lipids, then they should be more accessible to quenching by dynamic quenching due to a more open surface. We thus measured the quenching of gramicidin Trps by KI in 0, 25 and 50 mol% DMPE bilayers. We find that although the presence of PE lipids consistently resulted in a small shift of the emission to higher energies, presumably reflecting the more nonpolar character of PE surfaces, PE resulted in both a higher quenching coefficient and a greater overall quenching of the Trp residues: KI concentrations of 0.5M or greater quenched 75.4 ± 0.5% of gramicidin in 100% DMPC, but 78.5 ± 1.1% in 75:25 PC:PE and 84 ± 1.1% in 50:50 PC:PE. Thus, the presence of PE lipids allows for greater accessibility of KI to Trp residues.

4. Discussion

The effects of PE lipids on the conformational stability of membrane proteins has not yet been fully explored. Membrane proteins, in particular transporters, are thought to shuttle between conformational states and these states can be maintained by strong interactions between the lipid head groups and the interfacial residues of the protein [24,25]. It is

reasonable to believe that changes in lipid phase and composition may stabilize a particular protein conformation and thus alter protein function. In this paper, we have focused on the role of PE lipids in stabilizing protein–lipid contacts.

PE lipids have been shown to profoundly affect the physical properties of membrane surfaces due to their tendency to induce strain and increase the spontaneous curvature of bilayers [26,27]. Molecularly, PE lipids have smaller head group cross-sectional areas than PC lipids and under most biological conditions, the head group area has a smaller diameter than the hydrocarbon chains. It is assumed that the smaller head groups allow for closer packing and increases the spontaneous curvature, thereby promoting inverse hexagonal phases in which the higher spontaneous curvature is expressed as a physical curvature.

One of the parameters we have used to spectroscopically correlate to the spontaneous curvature in bilayers, the angular ratio, $\boldsymbol{B}$, is related to the packing of the lipid molecules in the membrane. This parameter is determined from the ratio of the rotational amplitude, which is related to the free volume, of a probe located close to the membrane surface to a more deeply buried one [14]. As detailed in the results section, we have used $\boldsymbol{B}$ to characterize a quantity we call pseudo-curvature upon certain perturbations to the membrane. In the case of oleoyl PE/PC lipids, the spontaneous monolayer curvature and the pseudo-curvature are identical by definition. In the absence of either direct measurement or better knowledge of the molecular interactions involved, we cannot be certain that $\boldsymbol{B}$ correlates uniquely with monolayer spontaneous curvature, so we use the name pseudo-curvature to remind the reader that we are dealing with a correlation, and not a direct measurement, to a spontaneous curvature. The changes in $\boldsymbol{B}$ seen with increased PE content, gramicidin incorporation, temperature and pressure qualitatively fit well with a spontaneous curvature and makes sense in terms of simplified geometrical interpretations (e.g., [28,29]): The decrease in $\boldsymbol{B}$ with PE content is consistent with a more cohesive in-plane tension close to the surface relative to deeper in the hydrocarbon region. Its reversal with gramicidin content is consistent with a decrease in spontaneous curvature due to the incorporation of a species into the membrane that exerts a complementary set of in-plane tensions. Raising the temperature increases the discrepancy between the in-plane tensions of hydrocarbon chains and the lipid head groups, whereas pressure reverses this effect.

The second method we have used to correlate to curvature is based on the physical properties of the membrane surface. The emission energy of Laurodan is highly sensitive to the polarity of its environment [16]. The shift in the center of mass of the emission spectrum with increasing PE content reflects a decrease in surface hydration, consistent with previous reports. Like the angular ratio, the shift in emission energy of Laurodan tracks the changes in curvature well with regards to PE content, GR content, temperature and pressure. Thus, the hydration and/or polarity of the membrane surface changes concomitantly with pseudo-curvature. As presented in the results section, Laurodan emission serves as an alternate correlate to a pseudo-curvature, although it is unclear whether the correlations through the Laurodan and stearic acid probes are equivalent.

To determine the effect of PE lipids on protein-lipid contacts, we have used gramicidin as a model system since the lipid interactions this peptides have been characterized [30]. The hydrogen bonds formed between the indole protons and presumably the lipid carbonyls can be ruptured by increased temperature and pressure. Loss of these contacts can be followed by the increase in rotational motion of the indoles as seen by a decrease in fluorescence anisotropy [5]. We find that increasing the PE content of the membrane increases the rotational motion consistent with a loss of strong peptide-lipid interactions. In the absence of PE, increasing the temperature results in an increase in rotational motion towards the values seen in the presence of PE. In the absence of PE, the application of pressure increases the rotational motion of the Trp residues due to a rupture of hydrogen bonds towards the atmospheric values in the presence of PE. In the presence of PE, the rotational motion decreases similar to probes lacking hydrogen bond donors that do not form links with the lipid carbonyls. If we assume that all contacts are lost at 50 mol% PE, we can calculate the enthalpy involved in these associations for the temperature data. We find that it is very low ($\sim$ 2 kcal/mol). Although the reason for the loss in indole–lipid hydrogen bonds

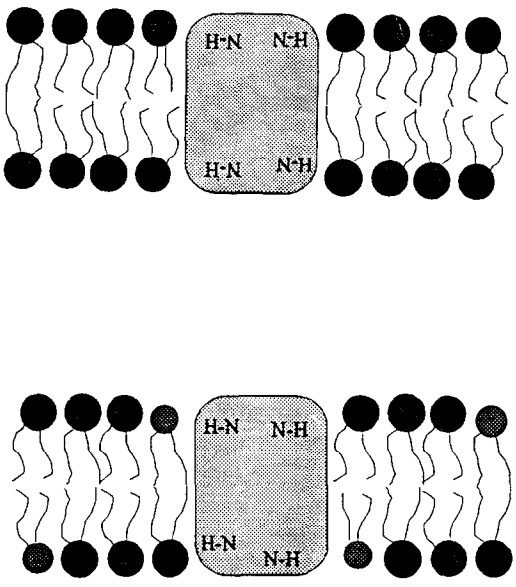

Fig. 9. Cartoon depicting the distance between PC head groups (lipids with larger head groups) and gramicidin (rectangle) versus the distance between PE head groups (smaller head groups) and gramidicin.

due to PE is unclear, we speculate that it may be caused by an increase in the distance between the gramicidin indoles and the PE head groups due to either the smaller size of the PE head groups or the closer association of the PE lipid head groups at the expense of PE–gramicidin as depicted in Fig. 9. This greater distance between the indoles and the PE molecules correlates with the greater accessibility of aqueous quenchers to these residues. This increased distance or PE self-association does not necessarily need to be great to overcome the low energy associated with the loss of these interactions.

In conclusion, we have developed spectral methods to easily view changes which correlate to spontaneous curvature associated with various perturbations in oleoyl PE/PC systems. The correlations were then extended to the myristoyl PE/PC which were more suitable for gramicidin measurements. Our results suggesting that PE lipids may affect protein function by altering protein–lipid contacts seem reasonable and await further confirmation using other techniques. We note that PE lipids may also regulate membrane protein function by other mechanisms, such as direct hydrogen bonding to the proteins. In these complicated systems, it is likely that the combination of several factors are at work.

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