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A model for gramicidin A′-phospholipid interactions in bilayers

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Abstract. A model is proposed for the effect of gramicidin A′ on the order and structure of phospholipid dispersions. According to this model, the addition of gramicidin A′ influences the surrounding lipids via two independent mechanisms. The first arises from a drop in surface pressure for those lipids substantially bounded by gramicidin A′. The second mechanism arises from the increase in the phospholipid headgroup spacing due to the small polar region of the polypeptide. The model provides an explanation for the currently available NMR, X-ray diffraction and Langmuir monolayer results. The model also suggests mechanisms for the ability of gramicidin A′ to trigger a transition of the lipid from the lamellar to hexagonal II phase, the dependence of this transition on the lipid chain length and the formation of a lamellar phase with lysophosphatidylcholine.

Key words: Lipid-protein interactions, gramicidin A′, model membranes

Introduction

Studies of lipid dispersions containing small hydrophobic polypeptides, such as gramicidin A′, yield information which may be useful in understanding the nature of lipid-protein interactions in biological membranes. Gramicidin A′ is often cited as a model system for the hydrophobic helical stems of membrane-associated proteins (Chapman et al. 1977; Cornell et al. 1978; Rice and Oldfield 1979; Rajan et al. 1981; Cortijo and Chapman 1981; Cortijo et al. 1982; Cornell and Keniry 1983; Lee et al. 1984; Tanaka and Freed 1985; Killian and de Kruijff 1985; Killian et al. 1985a and b; Brasseur et al. 1986; Cornell et al. 1988a). There is now general agreement that gramicidin A in phospholipid bilayers predominantly exists as a π_{LD} helical dimer which partitions into the hydrophobic region of the bilayer (for a recent review see Cornell 1987).

In this communication we present a theoretical analysis which although crude, provides a first approximation to explaining the interaction of gramicidin A′ with phospholipid dispersions.

Abbreviations: NMR: nuclear magnetic resonance; DMPC: dimyristoylphosphatidylcholine; S: molecular order parameter; CSA: chemical shift anisotropy; DPPC: dipalmitoylphosphatidylcholine; LPC: lysophosphatidylcholine

The model

The analysis is based upon the view that the equilibrium area of a phospholipid molecule in a bilayer is due to a balance of forces: those forces F_1 acting on the heads and chains with a net effect tending to increase the area per molecule, and those forces, F_2 acting at the interface and elsewhere in the molecule tending to decrease the area per molecule.

In a bilayer it is difficult to obtain a direct measure of these opposing forces. However, results from monolayers may be interpreted to give the approximate form of F_1. Unlike bilayer dispersions in bulk water, the phospholipids at an air-water interface may direct their hydrocarbon groups out of the water and minimise their interfacial energy over a range of packing densities. Thus when compressing such a monolayer from the gaseous to condensed state, the work done in ordering the lipid includes all of the lateral electrostatic, entropic and dispersive based forces between lipids and provides an empirical measure of the form of F_1.

As a first approximation, and by analogy with the pi-A curves typically obtained from phospholipid monolayers we have chosen to describe the force-area relationship for F_1 as a hyperbola, i.e.: $F_1 = K_1/A$, where A is the area available to the polar and nonpolar groups per molecule in the monolayer and K_1 is a constant.

The major opposing lateral force within the membrane, F_2 is generated at the interface and is related to the fall in surface tension with the formation of the membrane. The surface pressure within an air-water monolayer film is the difference between the surface tension of pure water and the surface tension of the film. For small variations about equilibrium, this force is taken to be linearly related to the interfacial area per molecule, i.e.; $F_2 = -K_2 A$ where A is the interfacial area per molecule and K_2 is a constant.

This balance of forces is shown in Fig. 1 and depicts the balance of forces per unit length along a line within the plane of the membrane. At this stage the membrane is assumed to be infinitely thin. The expansive force is arbitrarily shown as positive and the compressive force as negative. The equilibrium area is determined when the net force is zero due to a cancellation of the opposing effects. Under these conditions $F_1 = -F_2$ and thus $K_1/A = K_2 A$ yielding $A_{\text{equilibrium}} = (K_1/K_2)^{1/2}$. The force F_1, is separated into contributions from the headgroup, $F_{1h} = K_{1h}/A_h$ and from the chains, $F_{1c} = K_{1c}/A_c$. A_h and A_c are the areas per molecule available to the lipid headgroups and chains respectively. The concept of dividing the lateral pressure into contributions from the polar and nonpolar regions of the phospholipid can be compared with the liquid crystal approach of Petrov and Bivas (1984).

The effect of adding gramicidin A′ to a phospholipid bilayer is seen as arising from two mechanisms. The first is that the peptide favours the hydrocarbon interior of the membrane and contributes little to the packing of the lipid headgroups. The second is that addition of gramicidin lowers the bilayer surface pressure. Let us consider these two assumptions in turn.

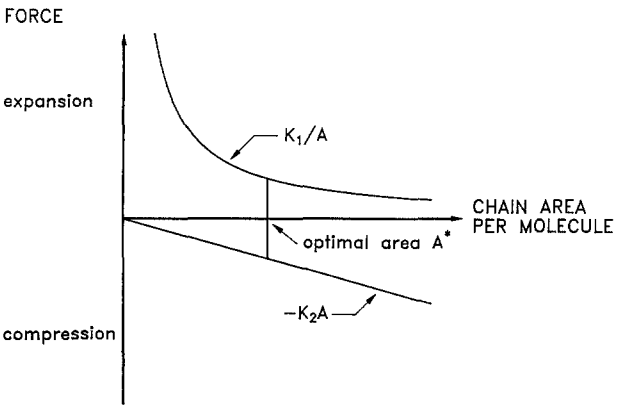

Fig. 1. Schematic representation of the balance of forces which determine the optimal area of the hydrocarbon chain in a lipid bilayer. The force tending to expand the area per molecule derives from the entropic effects of the headgroups and the chains, the steric interaction of the hydration shells surrounding the headgroups, electrostatic effects and steric effects arising from the chains. They have all been grouped into a common term K_1/A. The force tending to compress the area per molecule derives from the entropic exclusion of the water from the hydrocarbon regions, from the van der Waals attraction of the chains and any residual attractive electrostatic effects. These have been grouped into a common term $K_2 A$

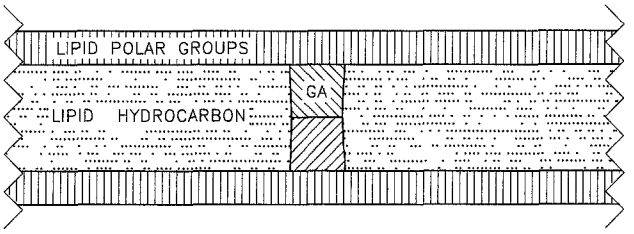

Fig. 2. When gramicidin A′ enters the bilayer the small size of its polar ethanolamine group causes the surrounding lipid headgroups to close over the ends of the cylindrical dimer producing an increase in the area available for the lipid headgroup reorientation

Reduction in headgroup packing

There are a number of reports that suggest that gramicidin A′ incorporates into the hydrocarbon region of the lipid (in particular, Weinstein et al. 1979, 1985). As a consequence of the absence of a polar group on gramicidin A′ this location causes an increase in the area available to the lipid headgroups which, it is argued will now sweep over a greater area. The essence of this assumption is shown in Fig. 2. On addition of gramicidin A′ the area available to the lipid headgroup is increased and thus F_{1h}, the expansive force contribution of the heads is reduced. This leads to $A_{\text{equilibrium}}$ moving to smaller values, in turn causing a reduction in the area and order of the lipid chains.

When formulating a quantitative description of the above effect the headgroup force constant, K_{1h}, is substituted by $K'_{1h}(1+\alpha R)^{-1}$. Where R is the mole ratio of gramicidin A′ to phospholipid, K'_{1h} the headgroup force constant for a lipid monolayer without gramicidin A′ and α is the ratio of the area of a gramicidin A′ molecule to a phospholipid molecule in a bilayer. An alternative view of this modification is that the available area per headgroup is now $A_{\text{equilibrium}}(1+\alpha R)$.

Reduction in surface pressure

The assumption that the addition of gramicidin A′ causes a reduction in the surface pressure at the hydrocarbon-water interface is taken from the pressure-area curves for monolayers of gramicidin A′ with phosphatidylcholine (Davion-Van Mau et al. 1987; Cornell et al. 1978). The molecular origin of this reduction stems from a combination of the packing geometry and polarity of the groups on the gramicidin A′ which lie in the region of the interface. The reduc-

tion in surface pressure is included here as an empirical concentration dependence of K_2 of the form

$$K_2'[(1-\psi)(1-6R)/(1+3.13R)+\psi],$$

where ψ is the ratio of the force constant, K_2 for those lipids adjacent to gramicidin A′ relative to the force constant K_2' of a pure phospholipid. Again R is the mole ratio of gramicidin A′ to phospholipid. The derivation of this expression is given in Appendix I. The origin of the $6R$ in the numerator is the number of phospholipids adjacent to each gramicidin A′ and the $3.13R$ in the denominator is the area ratio of gramicidin A′ to dimyristoylphosphatidylcholine (DMPC). A fall in surface pressure due to the effect will cause $A_{\text{equilibrium}}$ to move to larger values resulting in larger areas and disorder for the lipid chains.

Considering the combined effect of gramicidin A′ on both K_{1h} and K_2 suggests an initial reduction in lipid chain area for low concentrations followed by an increase in chain area at high concentrations of gramicidin A′. A corollary to this effect is that the molecular order parameter S_{mol} will first increase and then decrease with increasing concentrations of the polypeptide.

According to these assumptions the balance of forces is given by:

$$K_{1h}/(A_h-A_{hl})+K_{1c}/(A_c-A_{cl})=K_2(A_c-A_{cl}), \qquad (1)$$

where K_{1h} is the expansive headgroup force constant, K_{1c} is the expansive chain group force constant, K_2 is the compressive force constant which is dominated by the surface pressure at the interfacial region, A_h is the area of the lipid headgroup, A_c is the area of the lipid chain at the glycero-hydrocarbon region, A_{hl} is the limiting area of the lipid polar groups under maximal compression, A_{cl} is the limiting area of the lipid chains under maximal compression.

Expression (1) is a balance of forces per unit length along a line within an infinitely thin membrane. It may equally well describe a balance of pressures acting over the cross-section of a membrane of finite thickness. Expression (1) includes the refinement of allowing for the limiting areas of the heads and chains in the expressions for F_1 and F_2.

Rearranging (1) in terms of the area per lipid hydrocarbon region, A_c gives:

$$\begin{aligned}A_c^2-A_c[(2K_2A_hA_{cl}+K_{1h}-2K_2A_{cl}A_{hl})/(K_2A_h\\-K_2A_{hl})]+(K_{1h}A_{cl}-K_{1c}'A_h+K_{1c}'A_{hl}\\+K_2A_{cl}^2A_h-K_2A_{cl}^2A_{hl})/(K_2A_h-K_2A_{hl})\\=0\end{aligned} \qquad (2)$$

The primed K_{1c}' denotes the force constant of a gramicidin A′-free bilayer. The least constrained unknown in the model is the ratio of force constants K_{1c}'/K_{1h}' for a gramicidin A′ free bilayer. This ratio together with the known total lateral pressure of a gramicidin A′ free bilayer gives an estimate of the individual force constants K_{1c}' and K_{1h}'. To obtain reasonable agreement with the data, the present model requires that the polar group force constant dominates the lateral pressure in gramicidin A′-free bilayers. This is consistent with the observation of many authors that hydration plays a major role in determining the structure of lipid dispersions (Smaby and Brockman 1985; Kirk and Gruner 1985; Ljunggren and Eriksson 1985; Ben-Shaul and Szeifer 1985; Killian and de Kruijff 1985b). In the present treatment good agreement between the model and the experimental results was obtained for a ratio of $K_{1h}'/K_{1c}'=5$. Justification for a value of this order may be seen in a comparison of the surface free energy γA, and the free energy of transfer of a hydrocarbon molecule, equivalent to the DMPC chains, from water to liquid hydrocarbon. Using Tanford's (1980) estimate of the chain free energy of 0.7 kcal/CH_2 we obtain approximately 6×10^{-21} J/molecule for the DMPC chain free energy. The surface free energy is near 2×10^{-20} J/molecule. This gives a ratio of 3–4 for the two energies which is of the same order used in the model. The ratio is of course a comparison of forces or pressures and not of free energies, however, for small fluctuations around $A_{\text{equilibrium}}$ the ratios should be similar.

Substituting for the concentration dependence of K_{1h} and K_2,

$$K_{1h}=K_{1h}'/(1+\alpha R)$$

and

$$K_2=K_2'[(1-\psi)(1-6R)/(1+3.13R)+\psi]$$

the expression for chain area of the lipid, A_c may be solved in terms of R and a set of constrained constants.

The sum of the headgroup and chain pressures for a gramicidin A′-free monolayer are taken as equal to 25 dyne/cm. The ratio, ψ, of the surface pressure of a lipid adjacent to a gramicidin A′ molecule to that of a pure lipid is taken from the experimental value for a monolayer of gramicidin A′ at an area of 20 nm^2 per molecule, i.e., 9 dyne/cm (Cornell et al. 1978).

Figure 3 shows the effect on A_c of the variation in R from zero to 0.2. The approximation is made that K_{1h} and K_2 are independently affecting either the heads or the glycerohydrocarbon region of the molecule and K_{1c} is assumed to be constant and equal to K_{1c}'. It is also assumed that rapid molecular exchange between all of the environments in the bilayer produces a common perceived area and thus an averaged order when studied by NMR. We now compare predictions of the model with the available experimental data.

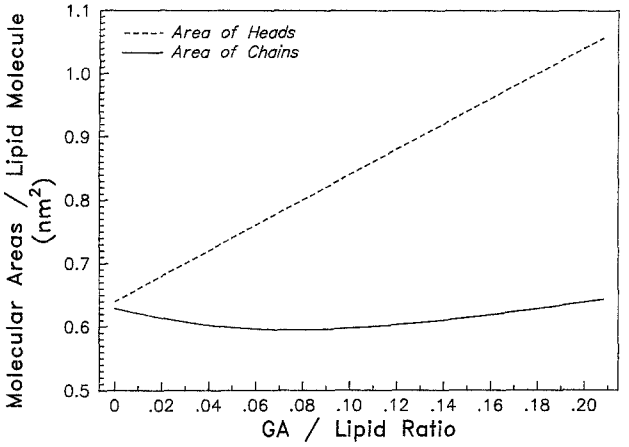

Fig. 3. The area per lipid headgroup and per hydrocarbon unit predicted by the expression $A_{\text{equilibrium}}(1+\alpha R)$ and expression (2) in the text. The parameters used to obtain this curve were: $r=P_c/P_h=0.20$, $\alpha=3.13$, $\psi=0.40$, $A_{hl}=0.565\ \text{nm}^2$, $A_{cl}=0.42\ \text{nm}^2$, $A_{\text{equilibrium}}=0.64\ \text{nm}^2$ and $P_m=5.0\ \text{nm}^{-1}$. The constants K'_{1h}, K'_{1c} and K'_2 were calculated via $P_m(A-A_{hl})/(2+2r)$, $P_m(A-A_{cl})/(2+2/r)$ and $P_m/2(A-A_{cl})$ respectively. The value of $r=0.20$ is chosen for $R=0$ and then becomes a dependent variable of the model

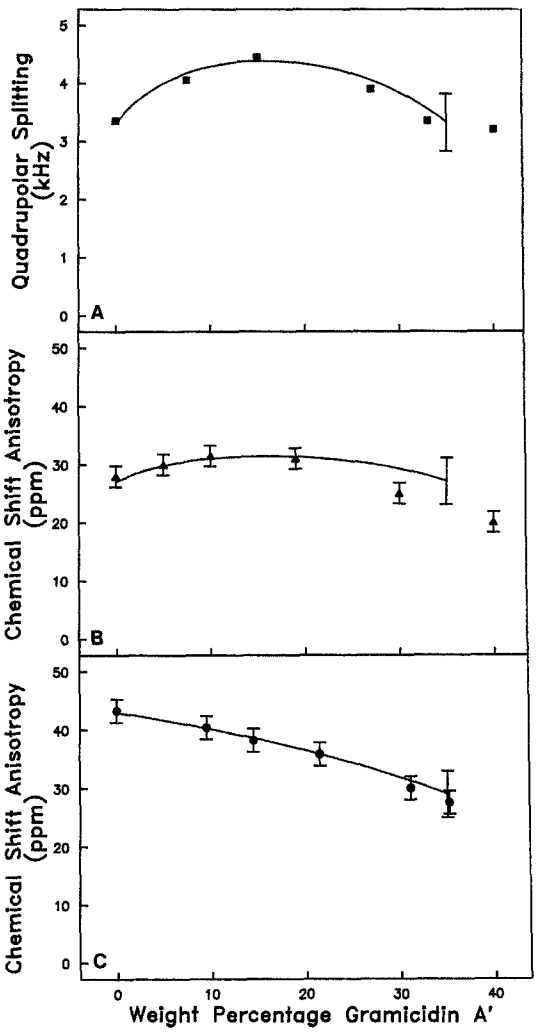

Fig. 4A–C. The variation of phosphorus-31 and carbon-13 CSA of the phosphate headgroup and *sn*-1 carbonyl, and the variation of the deuterium-2 quadrupolar splitting of the terminal methyl of the hydrocarbon chains of DMPC as a function of the mole ratio of gramicidin A' per DMPC molecule. The phosphorus-31 CSA were taken from Cornell et al. (1988 b), the carbon-13 CSA, from Cornell and Keniry (1983) and the deuterium-2 data from Rice and Oldfield (1979). The smooth curves denote the semi-empirical predictions of the model. The error bars at the end of each curve arise from the cumulative uncertainties in determining $d\Delta\nu/dT$ and dA/dT

Solid state NMR

Figure 4 shows a summary of the effect of gramicidin A' on the phosphate, carbonyl and chain regions of DMPC. The deuterium data is from Rice and Oldfield (1979) and the carbonyl data from Cornell and Keniry (1983). The phosphorus data was obtained from Cornell et al. (1988 b). When analysing this data we used the Peterson and Chan (1977) approximation in which the observed NMR splittings ($\Delta\nu$) are assumed proportional to the product of two factors, S_{conf} and S_{mol}, i.e.,

$$\Delta\nu \propto S_{\text{conf}} \cdot S_{\text{mol}},$$

where S_{conf} is a consequence of the order and conformation within a molecular fixed reference frame, and S_{mol} results from the order of the entire molecule in a laboratory fixed reference frame. In order to relate the area changes seen in Fig. 3 to the NMR data it is further proposed that the primary effect of this change is to modify S_{mol} and that conformational changes within the molecular frame do not contribute to the concentration dependent NMR splittings seen in Fig. 4. The narrow peak from the *sn*-2 carbonyl group (Cornell and Keniry 1983) supports this approach. Should the changes seen in the carbonyl data arise from conformational alterations, this peak would be expected to broaden. This approach is further justified by the qualitatively similar behaviour of the carbon-13 chemical shift anisotropy (CSA) of the *sn*-1 carbonyl group relative to the deuterium labelled sites along the hydrocarbon chain. Given that the orientation of the interaction is very different for the deuterium and carbon nuclei it is unlikely that a conformational change in the lipid would result in a similar concentration dependence of the NMR splittings.

The remaining step in the argument is to identify the form of the dependence of S_{mol} on molecular area. This is achieved empirically using the results of Trahms and Klabe (1985), Davis (1979), and data obtained in Cornell et al. (1988 b). These data are used to estimate $d\Delta\nu/dT$ for the phosphorus-31, the terminal methyl deuterons and the *sn*-1 carbonyl carbons over the initial 10 °C above the phase transition tem-

Table 1. The differentials $d\Delta\nu/dT$ for the deuterium quadrupolar splittings of the terminal methyl of DPPC, the phosporus-31 CSA of the DPPC phosphate group and the carbon-13 CSA of the *sn*-1 carbonyl group over the initial 10 °C above the $P_{\beta'}$-L_α phase transition temperature. These data were taken from Davis (1979), Trahms and Klabe (1985) and Cornell et al. (1988 b). From Janiak et al. (1979) dT/dA was determined to be 390 °C/nm^2 yielding the values of $d\Delta\nu/dA = (d\Delta\nu/dT) \cdot (dT/dA)$.

	^{2}H	^{31}P	^{13}C
$d\Delta\nu/dT$	0.06 kHz/° C	0.11 ppm/° C	0.25 ppm/° C
$d\Delta\nu/dA$	24.2 kHz/nm^2	35.2 ppm/nm^2	99.0 ppm/nm^2

perature of dipalmitoyl-phosphatidylcholine (DPPC). Likewise using X-ray diffraction data of Janiak et al. (1979) we obtain dA/dT over the same range of temperature. Using these two data sets we derive: $d\Delta\nu/dA = d\Delta\nu/dT \cdot dT/dA$. The values of $d\Delta\nu/dT$ and $d\Delta\nu/dA$ used in the calculations are shown in Table 1. Thus from the dependence of chain area A_c on gramicidin A′ concentration shown in Fig. 3 we obtain the concentration dependence of the static splitting for the three nuclei as shown in Fig. 4. The agreement is encouraging and prompts further exploration of the model.

One obvious shortcoming in this approach is that a component of the reduction in $\Delta\nu$ will result from thermally induced disorder which is independent of the molecular area in the membrane. By restricting our estimates of $d\Delta\nu/dT$ and dA/dT to the initial few degrees above the transition temperature we hope to minimise this contribution. A further assumption that we have been obliged to introduce is that the above differentials, which were obtained for DPPC, apply to DMPC. The results of Evans and Needham (1986) show very similar values for dA/dT of 390 °C/nm^2 for DMPC at 30 °C

X-ray diffraction

At a concentration of approximately 1 : 6 gramicidin A′ molecules per phospholipid the area per polypeptide-lipid unit is 0.91 nm^2 (Cornell et al. 1988 b). The model predicts a lipid area, A_c, which is essentially unaltered from the pure lipid value of 0.64 nm^2. This suggests an area per gramicidin A′ molecule of 2.4 nm^2. This may be compared with the area per molecule derived from the single crystal study reported by Koeppe et al. (1978) of approximately 2 nm^2. Even allowing for the larger area per molecule expected for a two dimensional fluid rather than a crystalline film, the X-ray diffraction results of 2.4 nm^2 per gramicidin A′ molecule is consistent with a fall in the average surface pressure of the mixed bilayer. Comparison with the monolayer study by Cornell et al. (1978)

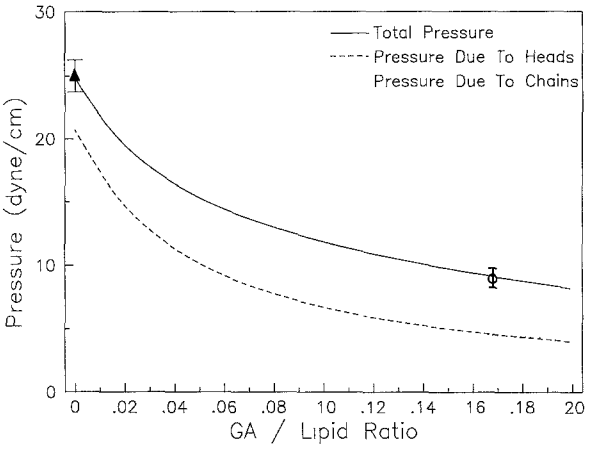

Fig. 5. The calculated pressure due to the lipid headgroups and to the lipid hydrocarbon chains as a function of the gramicidin A′ to lipid ratio. The surface pressure is in units of force per unit length of the edge of the surface. Thus the head and chain pressures represent the total force on the heads and the total force on the chains per unit length of the membrane edge. At a ratio of 0.18–0.20, P_c exceeds P_h which we propose triggers a transition from the L_α to H_{II} phase. The *full triangles* and *open circles* denote the results of monolayer experiments on DMPC by Phillips and Chapman (1968), and a combination of the results of Cornell et al. (1978) on a 15.1 mol% dispersion of gramicidin A′ in egg yolk phosphatidylcholine and the X-ray diffraction results from Cornell et al. (1988 b). The X-ray diffraction results were used to determine the unit area at a concentration of 1 : 6 and the monolayer study to determine the pressure

shows the surface pressure needs to have fallen below 10 dyne/cm before the area of the gramicidin A′ monolayer exceeds 2.0 nm^2 per molecule.

Further support for a fall in surface pressure with the addition of gramicidin A′ may be seen in comparing the X-ray results with the mixed monolayer studies of the same report.

Monolayer studies

An integral part of the present model is the fall in K_{1h} and K_2 as the gramicidin A′ concentration is increased. Using the area changes shown in Fig. 3, we may predict the fall in total surface pressure $P_m = (P_h + P_c)/2$ as shown in Fig. 5 (top curve). The factor of 2 allows for the comparison being made with a monolayer and not a bilayer. The points for pure lipid are taken from Phillips and Chapman (1968). Using the monolayer data for a 1 : 6 (15%) dispersion of gramicidin A′ in lipid (Cornell et al. 1978), the X-ray determined area for a gramicidin-lipid unit of 0.91 nm^2 indicates a surface pressure of 10–12 dyne/cm. As seen on Fig. 5 this drop in surface pressure is consistent with the prediction of the model.

A further observation from the mixed monolayer studies of gramicidin A′ and lipid is the apparent con-

densing effect of the gramicidin A′ on the area of the surrounding lipid. This effect was observed for varying ratios of gramicidin A′ in lipid but at *constant surface pressure*. To simulate the monolayer result at constant pressure it is necessary to correct for the drop in surface pressure at each gramicidin A′ concentration. With this correction the lipid area is always condensed relative to a pure monolayer.

A final observation that was previously unexpected is the small area per gramicidin A′ molecule of 1.38 nm^2 in the pure monolayers of gramicidin A′ at pressures expected in a pure lipid bilayer. The present model explains these areas as a consequence of the high surface pressures employed in their measurement.

Lamellar to hexagonal phase transitions

A further aspect of the model is the predicted change in the relative contribution of the headgroup pressure and chain pressure to the total surface pressure of the membrane. It is proposed that the ratio P_c/P_h plays a role in determining the phase stability of the dispersions. We suggest that should the headgroup pressure dominate, the lipid will tend to form bilayers or micelles depending on the *geometrical* constraints dictated by the hydrocarbon chains (Israelachvili et al. 1980; although these authors base their theory on a balance of free energies rather than pressures). Should the chain pressure dominate the curvature is reversed and the lipid will tend to form a reverse hexagonal phase. We have neglected the different thicknesses of the polar and non-polar layers of each monolayer and have chosen simply to balance the polar and non-polar pressures per unit edge of the monolayer. The dependence of P_h and P_c on polypeptide concentrations are shown separately in Fig. 5. The contribution of the heads and chains are nearly equal above $R=0.1$. Small changes in $r=P_c/P_h$ will cause either P_c or P_h to be greater. Using the same constants that were used to generate the smooth curves shown in Fig. 4, the cross over from P_h dominating to P_c dominating occurs at $R=0.18$, in broad agreement with the disruption of the L_α phase seen from the density gradient and X-ray data. The value of r at $R=0$ is 0.2, indicating that the headgroups dominate in establishing the surface pressure in a pure lipid bilayer, i.e.: as discussed above the dominant contribution to the two dimensional geometry of a fluid phase phospholipid is the hydration force between the phospholipid polar groups.

Another test of this hypothesis is the predicted dependence of the disruption of the lamellar phase on the chainlength of the lipid. Using R_t to describe the concentration at which this disruption occurs, we have determined its dependence on chainlength by setting $P_{cn}=P_{c14}\cdot n/14$, where P_{cn} and P_{c14} are the chain pressures at chainlengths of n and 14 carbons respectively. By assuming that P_h is independent of the change in n, A_h and A_c are recalculated and thus the ratios of the pressures, P_c/P_h redetermined. A comparison of the predicted result and the available data is shown in Fig. 6. The data points are taken from the NMR results of van Echteld et al. (1981, 1982), the NMR data of Rice and Oldfield (1979), and the X-ray diffraction result of Cornell et al. (1988 b). The model shows a similar trend to the experimental data. The question of the range of concentrations over which this transition occurs is not addressed by this simple model. The study of Killian and de Kruijff (1985) suggests that the formation of the hexagonal II phase is associated with a phase separation of the polypeptide into gramicidin-rich and gramicidin-poor regions. Phase separation would give the appearance of a higher concentration of gramicidin A′ being required before the dispersion was totally transformed into the hexagonal II phase.

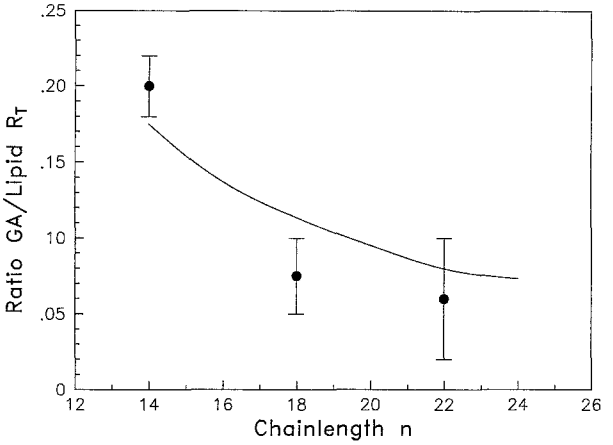

Fig. 6. The predicted and experimental (full circles) chainlength dependence of the critical concentration (R_t) of the L_α-H_{II} phase transition. The experimental data points were taken from Van Echteld et al. (1981) ($n_c=18$), Van Echteld et al. (1982) ($n_c=22$) and Cornell et al. (1988 b) ($n_c=14$). The predicted curve was obtained using the parameters listed in the caption to Fig. 6 and $r=0.20\times n_c/14$

A further challenge for the model is the observation by Killian et al. (1983) and Spisni et al. (1983) that lysophosphatidylcholine (LPC) forms a lamellar phase when dispersed with high concentrations of gramicidin A′. By treating LPC ($n=16$, where n is the number of carbons) as equivalent to a diacyl lipid with $n=8$, we calculate that $r=P_c/P_h<1$ for all values of R. That is we expect a phase possessing a positive monolayer curvature such as an hexagonal I or micellar phase, or should the geometrical constraints dictate, a zero curvature as in a planar bilayer. The concentration of gramicidin A′ at which such a bilayer phase would form is easily calculated from the geometrical requirement (Israelachvili et al. 1980), that $v/a_0 l$ is in the range 1 to 0.8. Here v is the hydrocarbon volume of a

gramicidin A-phospholipid unit, a_0 is the area of such a unit at the membrane interface, and l is the hydrocarbon thickness of the monolayer. The physical basis of the Israelachvili et al. (1980) approach is essentially that the morphology of a phase may be determined by assuming a continuum average of its component molecules. Using a hydrocarbon volume for LPC of 0.63 nm^3 and for gramicidin A′ of 2.8 nm^3, an area for LPC of 0.60 nm^2 and for gramicidin A′ of 2.0 nm^2, and a monolayer hydrocarbon thickness of 1.5 nm, it is easy to calculate that R is approximately 0.30 for a $v/a_o l$ of 0.8. This is the same order of magnitude as the experimental value of 0.25 obtained by Killian et al. (1983). The origin of the effect is simply that the average inherent geometry of gramicidin A′ is such that $v/a_0 l$ is close to 1. Thus as it is added to the LPC, which has a geometry such that $v/a_0 l = 0.7$, this ratio is raised above the critical level of 0.8 which permits the formation of bilayers.

Conclusion

Based upon the polypeptide being located predominantly in the hydrocarbon interior of the bilayer this model provides a first approximation for the known effects of gramicidin A′ on lipid dispersion. The model accounts for the changes in the dynamic properties of the lipid as measured by phosphorus-31, deuterium-2 and carbon-13 NMR, and for the monolayer *pi-A* curves. The apparent condensing effect of gramicidin A′ on lipid monolayers, the disruptive effects of gramicidin A′ on the lamellar structure of lipid bilayers, and the ability of gramicidin A′ to form a lamellar phase with LPC is also explained.

The conclusions drawn from this study raise many questions concerning the origin of the physical effects of lipid interactions with proteins in biological membranes.

Appendix I

The effect of gramicidin A′ on the surface pressure (K_2)

Consider a lipid monolayer containing a mole ratio concentration, R=(mole of gramicidin A′/mol of phospholipid). Of the N_f potential sites available for lipids, N_0 are occupied by gramicidin A′, N_a are adjacent to gramidicin A′, and N_f are occupied by lipid free of adjacent gramicidin A′. The total number of sites N is thus $N_a + N_f + N_0$. If on average each gramicidin A′ molecule is adjacent to six adjoining lipids and occupies $3 \cdot 13$ lipid areas, then $N = 6N_g + N_f + 3.13 N_g$. Here N_g is the number of gramicidin A′ sites. Thus $N_f/N = (1-6R)/(1+3 \cdot 13R)$ where $R = N_g/(N_f + N_a)$. If it is assumed that the surface pressure of the free lipid sites N_f is K_2' and of the other sites K_2'', then $K_2 = K_2'(N_f/N) + K_2''(N - N_f)/N$, or if $K_2'' = \psi K_2'$, then $K_2 = K_2'[(1-\psi)[(1-6R)/(1+3.13R)) + \psi]$.

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