Intrinsic Curvature in Normal and Inverted Lipid Structures and in Membranes

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ABSTRACT The intrinsic or spontaneous radius of curvature, R_o, of lipid monolayer assemblies is expressed in terms of a lipid molecular packing parameter, V/Al, for various geometries. It is shown that the equivalent lipid length, l, in inverted hexagonal (H_{II}) phases, defined by a cylindrical shell of equal total lipid volume, yields an expression for R_o identical to that for inverted cylindrical micelles (or, equivalently, H_{II} phases in the presence of excess hydrocarbon). This identity is used to obtain values of the effective packing parameter for various phosphatidylethanolamines. The temperature dependence of the intrinsic radius of curvature is predicted to be negative and to be considerably greater than that for the lipid length in nearly all cases. The thermal expansion coefficient is not constant but is found to vary, depending on the value of the lipid packing parameter. A possible addition rule is constructed for the intrinsic radius of curvature of lipid mixtures, based on the linear additivity of the effective molecular volumes, V, and molecular areas, A. This relation is found to hold for mixtures of dioleoyl phosphatidylcholine (DOPC) with dioleoyl phosphatidylethanolamine, and a value of R_o of $\geq$95 Å ($V/Al = 1.08$) is obtained for DOPC. The energetics of the intrinsic curvature and lamellar-nonlamellar transitions are also discussed within the framework of the model.

INTRODUCTION

The concept of spontaneous (or intrinsic) curvature in membranes was introduced by Helfrich and used in the analysis of the shape of erythrocytes (Helfrich, 1973, 1974). In a membrane, a nonzero intrinsic curvature requires an asymmetry between the two surfaces of the membrane, which may be introduced, for instance, by an asymmetry in lipid composition or by a difference in the aqueous composition at the apposing surfaces of the membrane (cf. Evans and Skalak, 1980). By contrast, spontaneous curvature in a single monolayer can arise naturally from the molecular properties of the constituent lipids. This concept has been used extensively by Gruner and co-workers (Gruner, 1985; Kirk and Gruner, 1985; Gruner et al., 1988) in the analysis of the formation of inverted lipid phases, by using experimentally determined values of the intrinsic curvature. The latter were obtained from the water cylinder radii of inverted hexagonal (H_{II}) phases, in the presence of excess hydrocarbon solvent that was used to relieve the lipid chain packing restrictions inherent in H_{II} phases. Hui and Sen (1989) have also used similar methods in the treatment of the effects of lipid composition on the functional properties of various reconstituted membranes.

The principal source of the intrinsic curvature arises from the packing of the lipid molecules, as controlled by the intermolecular interactions. Israelachvili et al. (1976) have treated the lipid packing problem in terms of a chain packing parameter that is defined relative to the equilibrium area per lipid molecule at the polar-apolar interface, the size of which is derived from thermodynamic considerations. Here an analogous packing parameter is introduced, which differs from that used previously in that it is purely geometric and refers to the whole lipid molecule, rather than simply to the lipid chains in conjunction with the thermodynamically deduced interfacial area. The advantage for the present purposes is that the geometric packing parameter may be related directly to structural measurements derived from x-ray diffraction. The thermodynamic aspects of the lipid packing may then be deduced from the elastic curvature free energies. The geometric packing parameter is thus given by V/Al, where V is the volume of the entire lipid molecule, l is its length, and A is the area of the lipid headgroup at the lipid-water interface. When $V/Al = 1$, lamellar structures are formed, whereas normal (oil-in-water) and inverted (water-in-oil) curved structures are obtained for $V/Al < 1$ and $V/Al > 1$, respectively. This concept may be extended to include headgroup and chain interactions by scaling the effective lipid dimensions.

The packing parameter can be used to predict values for the curvature of different lipid structures, and this has been done by Hui and Sen (1989) for the mean intrinsic curvature of various lipid mixtures for one particular geometry. The purpose of the present work is to compare the predicted curvatures calculated for the range of different geometries found commonly to occur in lyotropic lipid polymorphism. This has been done for normal and inverted spherical and cylindrical micelles, for vesicles, for inverted hexagonal phases, and for membranes. Two different situations are distinguished: those in which the curvature is determined by the packing constraints, and those in which the packing constraints are released, e.g., by the addition of excess hydrocarbon, and the intrinsic curvature as defined experi-

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mentally by Gruner and co-workers (Gruner, 1985; Kirk and Gruner, 1985; Gruner et al., 1988) is obtained. The results are used to discuss the properties of the different systems. In particular, the temperature dependence is analyzed, the usefulness in constructing an addition rule for predicting the intrinsic curvature of lipid mixtures is assessed, and values for the effective packing parameter are derived from structural data in the literature.

METHODS

Within the geometric model, the intrinsic curvature predicted for a single lipid monolayer can be obtained from packing considerations according to Fig. 1. This is done in terms of the ratio of the lipid volume to surface area for the different structures (see Appendix). For (infinite) cylindrical micelles, in the absence of micellar packing restrictions that disturb the cylindrical symmetry (e.g., in H_{II} phases), the intrinsic curvature is given by

$$\left(\frac{1}{R_o}\right) = 2(V/Al - 1)/l, \qquad (1)$$

where R_o is defined as positive for inverted structures ($V/Al > 1$) and negative for normal structures ($V/Al < 1$), as can be verified easily by consideration of the two geometries (cf. Fig. 1, *A* and *B*, and Appendix). With a packing parameter $V/Al = 1$, planar structures that have zero curvature, $1/R_o = 0$, are obtained. For normal micelles, the minimum value of the packing parameter is $V/Al = 1/2$, corresponding to $R_o = l$ (cf. Fig. 1 *B*). Smaller values of the packing parameter require that the micelle core is filled, e.g., by hydrocarbon. For inverted micelles, the only restriction on the maximum value of the packing parameter is that *A* and, alternatively, the number of lipid molecules at the polar-apolar perimeter, shall not be unrealistically small. The predicted dependence of the intrinsic curvature on the effective packing parameter for normal and inverted cylindrical monolayer structures is given in Fig. 2. These results are also applicable to spontaneous cylindrical curvature of the complementary monolayers in lipid bilayers or biomembranes.

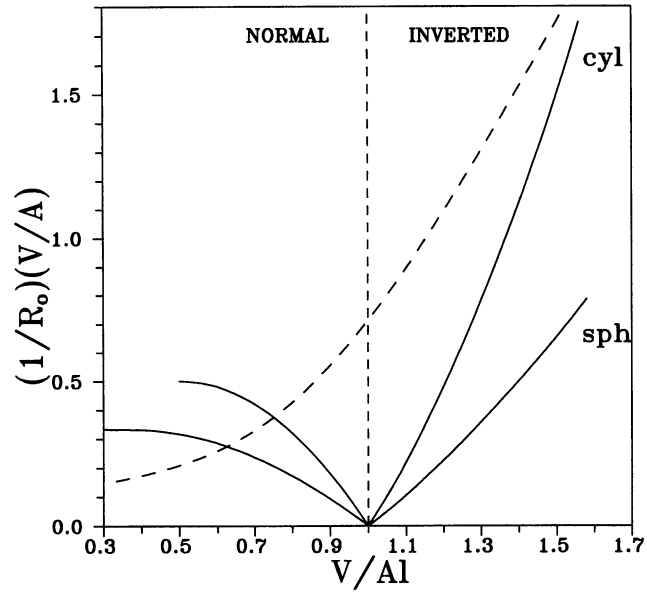

FIGURE 2 Absolute values of the normalized intrinsic curvature, $(1/R_o)(V/A)$, of cylindrical (cyl) and spherical (sph) lipid monolayer structures as a function of the packing parameter, V/Al, derived from Eqs. 1 and 2, respectively. Normal structures correspond to $V/Al < 1$, and inverted structures to $V/Al > 1$. The sign of the curvature of normal structures is opposite that of inverted structures. The dashed line represents the radius of the water cylinders in inverted hexagonal phases, derived from Eq. 3, where $l = l_{max}$. These remain inverted over the whole range of values given.

For spherically shaped monolayers, corresponding geometrical considerations show that the intrinsic curvature is given by (cf. Appendix)

$$\left(\frac{1}{R_o}\right) = \frac{2(V/Al - 1)/l}{1 + \sqrt{(4V/Al - 1)/3}}, \qquad (2)$$

where the same sign conventions for normal and inverted structures hold as for Eq. 1. For normal structures, the

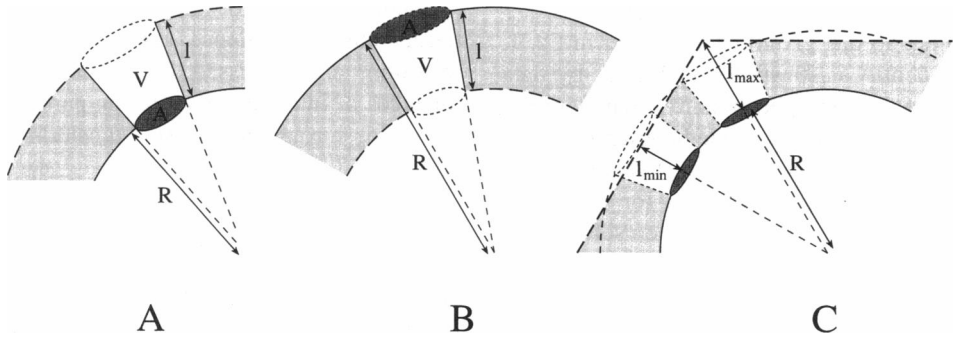

FIGURE 1 Topology of inverted (*A*) and normal (*B*) curved lipid monolayer structures, indicating the characteristic dimensions and specifying the monolayer curvature (*R*) and the lipid packing parameters (l and A). The corresponding parameters ($l = l_{max}$, l_{min}, and A) are defined for inverted hexagonal structures, with water cylinders of radius *R*, *C*. The surface to which *R* is measured is taken as the lipid-water interface of the monolayer. An alternative convention is to take the neutral plane (Rand et al., 1990), with corresponding redefinition of the characteristic lipid dimensions.

minimum value of the packing parameter is $V/Al = 1/3$, again corresponding to $R_o = l$, and a similar restriction holds for the maximum value in inverted structures as for cylindrical structures. The predicted dependence of the intrinsic curvature on the effective packing parameter for normal and inverted spherical monolayer structures is given in Fig. 2. These results are also applicable to the spontaneous spherical curvature of the complementary monolayers in lipid bilayers or biomembranes (cf. Hui and Sen, 1989).

For inverted hexagonal (H_{II}) phases formed by amphiphilic lipids in the absence of additional apolar components, the curvature is determined partly by the packing constraints of the hexagonal lattice, which destroy the cylindrical symmetry of the hydrocarbon chain region. The structure is unable to relax to the spontaneous curvature predicted by Eq. 1. The curvature of the water cylinders allowing for these geometric restrictions is given by (cf. Fig. 1 *C* and Appendix)

$$\left(\frac{1}{R_w}\right) = \frac{(1-c)/l}{cV/Al - 1 - \sqrt{(cV/Al)^2 - 2cV/Al + c}}, \quad (3)$$

where $c = 2\pi/(3\sqrt{3})$ for $l = l_{max}$, and $c = \pi/(2\sqrt{3})$ for $l = l_{min}$. (For the case $l = l_{min}$, real solutions are found only for $V/Al \geq 1.439$.) There is no unique length, l, for the lipid molecule in the H_{II} phase, because of the chain distortion caused by the hexagonal packing requirements. Here l_{min} is the minimum lipid length, measured along the direction joining the centers of the cylinders, and l_{max} is the maximum lipid length measured along a line 30° to the intercylinder axis (cf. Fig. 1 *C*). The dependence of the surface curvature in H_{II} phases on the lipid packing parameter is compared with that predicted for unconstrained cylindrical lipid structures in Fig. 2, for the case where $l = l_{max}$.

To eliminate the ambiguity of definition in inverted hexagonal structures, a characteristic lipid length, l_{eq}, can be defined for an equivalent cylinder (of inner radius R_w) in which the total lipid volume is equal to that in the H_{II} phase (cf. Eqs. A.2 and A.9 in the Appendix):

$$l_{eq} = (l_{min} + R_w)/\sqrt{c} - R_w, \quad (4)$$

where $c = \pi/(2\sqrt{3})$, corresponding to $l = l_{min}$ in Eq. 3. The expression for the curvature of the H_{II} phase given in Eq. 3 then becomes identical to that given for a cylinder in Eq. 1, with $l = l_{eq}$. This identity is illustrated in Fig. 3, by data from the H_{II} phases of different phosphatidylethanolamines and a phosphatidylcholine/phosphatidylethanolamine mixture. The figure gives some indication of the likely effective values for the lipid packing parameter, V/Al, for H_{II} phases of phosphatidylethanolamines. This should be useful in the analysis of the curvature of H_{II} phases in the presence of alkanes, for which the cylindrical model is more appropriate.

Currently there are no phases of biological lipids that are known to correspond to a regular three-dimensional arrangement of inverted spherical micelles. In principle, however, a treatment similar to that for the inverted hexagonal phase could be used. For packing of normal micellar structures the considerations are different. If water is assumed to be in excess, the micellar packing restrictions come only from the hydrocarbon region. For a system with $R_o > l$, a micellar structure without additional apolar material can only be achieved if the value of l is less than the unconstrained value. This is similar to the packing criterion considered by Israelachvili et al. (1976), where the length of the lipid chain must be less than the critical value corresponding to a fully extended, all-*trans* chain.

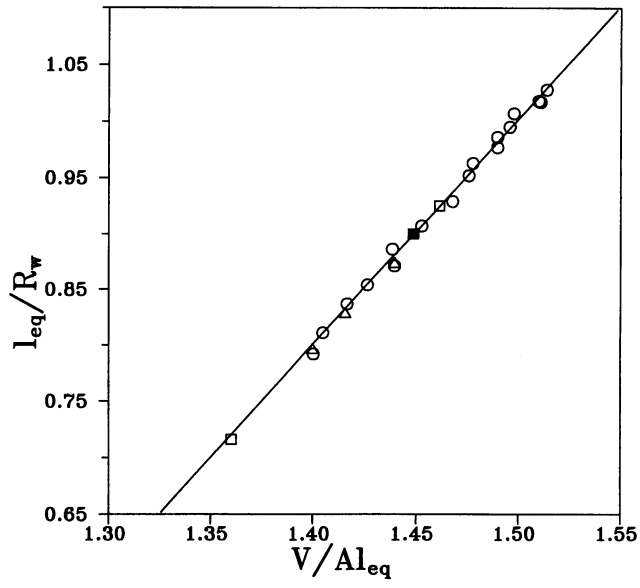

FIGURE 3 Dependence of the curvature, $1/R_w$, of the aqueous cylinders in H_{II} phases on the packing parameter, V/Al. The characteristic lipid length, l, is taken to be equal to the equivalent length, l_{eq}, defined by Eq. 4, both in calculating the packing parameter and in normalizing the curvature. ○, Data on DOPE at various temperatures (Tate and Gruner, 1989); △, data on DOPE/DOPC (5.07/1) (Tate and Gruner, 1989); □, data on didodecyl phosphatidylethanolamine (Seddon et al., 1984); ■, data on diarachinoyl phosphatidylethanolamine (Seddon et al., 1984). The straight line corresponds to the dependence predicted by Eq. 1.

RESULTS AND DISCUSSION

Structural aspects of the intrinsic curvature, namely the temperature dependence and the dependence on lipid composition, are discussed first. Then the energetic consequences of intrinsic curvature, in particular the lamellar-to-inverted hexagonal phase transition temperatures, are considered.

Temperature dependence of R_o

With increasing temperature, the lipid length, l, will shorten as a result of increasing chain rotational isomerism. By analogy with bilayers (cf. Cevc and Marsh, 1987), the change in lipid volume, V, is likely to be considerably smaller because of compensating changes in the area per molecule. In contrast to the situation for planar bilayers, the

change in A for curved structures is also likely to be small, because optimum headgroup packing can be maintained by adjusting the curvature. Because the two latter quantities appear as the quotient V/A in Eqs. 1–3, the effects of their limited expansion with temperature will further tend to cancel. In fact, the value of V/A was found to remain approximately constant over a wide range of temperature in the H_{II} phase of dioleoyl phosphatidylethanolamine (DOPE) (Tate and Gruner, 1989).

By differentiating Eq. 1 with respect to temperature, under the assumption that V/A remains constant, the temperature dependence of R_o for cylindrical micelles/monolayers is given by

$$\left(\frac{1}{R_o}\right)\left(\frac{dR_o}{dT}\right) = \left[2 + \frac{1}{V/Al - 1}\right]\alpha_l, \quad (5)$$

where $\alpha_l = (1/l)dl/dT$ is the expansion coefficient for the lipid length. Therefore, even if the expansion coefficient for the lipid length is approximately constant, as is likely to be the case, that for R_o (i.e., $\alpha_R = (1/R_o)dR_o/dT$) will vary with temperature as a result of the term in V/Al on the right-hand side of Eq. 5. The corresponding expression for the expansion coefficient of the intrinsic radius of curvature in spherical micelles, deduced from Eq. 2, is

$$\left(\frac{1}{R_o}\right)\left(\frac{dR_o}{dT}\right) = \left[2 + \frac{1}{V/Al - 1} - \frac{2V/Al}{4V/Al - 1 + \sqrt{3(4V/Al - 1)}}\right]\alpha_l, \quad (6)$$

and that for the radius of the water cylinders in H_{II} phases, obtained from Eq. 3, is

$$\left(\frac{1}{R_w}\right)\left(\frac{dR_w}{dT}\right) = \left[1 + \frac{cV/Al}{\sqrt{(cV/Al)^2 - 2cV/Al + c}}\right]\alpha_l, \quad (7)$$

where $c = 2\pi/3\sqrt{3}$ and $l = l_{max}$. In each case, the ratio V/A is again assumed to remain constant.

The dependence of the expansion coefficient for R_o on the lipid packing parameter, V/Al, is given for both cylindrical and spherical micelles in Fig. 4. The corresponding dependence for the expansion coefficient of the radius, R_w, of the water cylinders in H_{II} phases is also included in Fig. 4. The figure indicates that the absolute value of R_o (and of R_w) will decrease with increasing temperature for all accessible values of V/Al, because α_l is negative. The expansion coefficient for R_o diverges in both normal and inverted micelles at $V/Al = 1$, corresponding to the infinite radius of curvature of planar bilayer structures. The geometrical restrictions in H_{II} phases ensure that the expansion coefficient for R_w remains finite and greater than that for the lipid length for all values of l_{max}. As a result of the geometry of the curved micelles, the expansion in R_o is seen also to be considerably greater than that for the lipid length, except for normal micelles with $V/Al \leq 2/3$ for cylindrical structures and $V/Al \leq 0.6233$ for spherical structures.

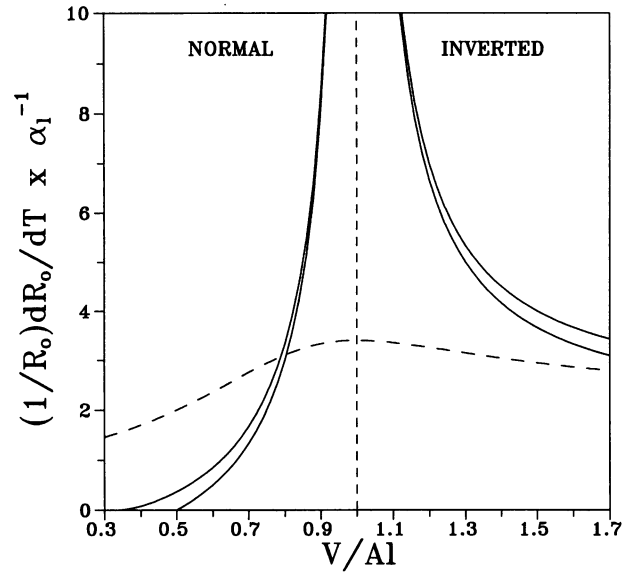

FIGURE 4 Absolute values of the expansion coefficient, $(1/R_o)dR_o/dT$, for the intrinsic curvature of cylindrical (*lower left-hand curve; upper right-hand curve*) and spherical (*upper left-hand curve; lower right-hand curve*) lipid monolayer structures as a function of the packing parameter, V/Al. Dependences are derived from Eqs. 5 and 6 for cylindrical and spherical geometries, respectively. Normal structures correspond to $V/Al < 1$, and inverted structures to $V/Al > 1$. The dashed line represents the expansion coefficient for the radius of the water cylinders in inverted hexagonal phases with respect to $l = l_{max}$, derived from Eq. 7. In each case the expansion coefficient for R_o is normalized with respect to that for the lipid length: $\alpha_l = (1/l)dl/dT$.

The experimental situation is illustrated in Fig. 5 for measurements of the temperature dependence of the radius of the water cylinders, R_w, in comparison with those for the equivalent lipid length, l_{eq}, in the H_{II} phase of DOPE (Tate and Gruner, 1989). The temperature dependence of l_{eq} is well fitted by the integrated equation for thermal expansion: $\ln(l/l^\circ) = \alpha_l \cdot T$, with a constant expansion coefficient: $\alpha_l = -0.0011°C^{-1}$. In contrast to the situation for l_{eq}, it is found that the temperature dependence of R_w cannot be fit adequately with a single, constant (i.e., temperature-independent) expansion coefficient. This result is in agreement with the predictions made previously from Eq. 5, which is appropriate in the present case. The best fit that can be obtained to the integrated equation for thermal expansion of R_w ($\ln(R_w/R_w^\circ) = \alpha_R \cdot T$), when assuming a constant expansion coefficient, α_R, is given in Fig. 5. The apparent expansion coefficient obtained under these assumptions is $\alpha_R = -0.0044°C^{-1}$, which is four times greater than that for the lipid length, α_l, and corresponds to an effective value of $V/Al_{eq} = 1.49$, obtained by using Eq. 5 for α_R. The latter value for the effective packing parameter lies within the range of those measured for the H_{II} phase of DOPE over the same temperature interval (cf. Fig. 3). For instance, the mean value of the lipid packing parameter calculated over this temperature range is $V/Al_{eq} = 1.47$, which from Eq. 5 would correspond to an effective mean expansion coefficient of $\langle\alpha_R\rangle = 4.15 \times \alpha_l$. Thus the steep temperature

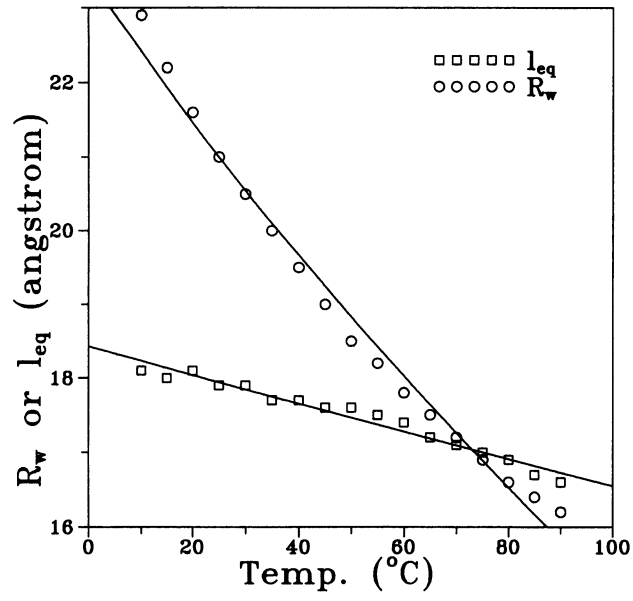

FIGURE 5 Temperature dependence of the radius, R_w, of the aqueous cylinders (○) and the equivalent lipid length, l_{eq}, defined by Eq. 4 (□), in the H_{II} phase of DOPE (Tate and Gruner, 1989). The solid lines represent the best fits to the temperature dependence, assuming constant coefficients of expansion, $\alpha_l = (1/l)dl/dT$ and $\alpha_R = (1/R)dR/dT$, as described in the text.

dependence of the spontaneous curvature can be explained by Eq. 5 in terms of a constant (and smaller) expansion coefficient of the equivalent lipid length (cf. Tate and Gruner, 1989).

Addition rule for R_o

A trial addition rule can be constructed for predicting the values of R_o for lipid mixtures on the basis of linear additivity of the characteristic dimensions, V, A, and l, of the individual lipid components. If X is the mole fraction of component 1, then

$$V = X \cdot V_1 + (1 - X)V_2 \quad (8)$$

$$A = X \cdot A_1 + (1 - X)A_2 \quad (9)$$

$$l = X \cdot l_1 + (1 - X)l_2, \quad (10)$$

where the subscripts refer to components 1 and 2 of the binary mixture, respectively. Equation 8 corresponds to conservation of volume, and Eq. 9 to preservation of the headgroup packing density. Equation 10 is somewhat less easy to justify, but, as will be seen below, it is not crucial for lipids with similar chain lengths. The Eqs. 8–10 may then be used in conjunction with Eq. 1 or 2 (or Eq. 3), depending on the particular geometry, to obtain a value for R_o. (It will be noted that this addition rule differs from that used by Hui and Sen (1989), who assumed linear additivity of the packing parameter, V/Al, which does not preserve conservation of volume and area.)

Rand et al. (1990) have measured the radii of the water cylinders in the H_{II} phase of dioleoyl phosphatidylethanolamine and phosphatidylcholine (DOPE/DOPC) mixtures, in the presence of excess tetradecane. These values should correspond to the unconstrained natural radius of curvature, R_o, and therefore Eq. 1 is the appropriate expression for the analysis. These data are given as a function of the mole fraction, X(DOPE), of DOPE in Fig. 6. It was found that the characteristic length of the lipid molecule, l (corresponding to l_{min} in this case), was essentially independent of lipid composition (Rand et al., 1990). A value of l = 15.5 Å is assumed here, corresponding to the value obtained for the DOPE/DOPC (3:1 mol/mol) mixture.* The values of V have been obtained from Eq. 8, using values of V_{PE} = 1206 Å^3 and V_{PC} = 1306 Å^3, which were obtained by summing the group contributions to the two lipids (see, e.g., Marsh, 1990). The result of a nonlinear least-squares fit, according to Eqs. 1, 8, and 9, is given in Fig. 6. It can be seen that the addition rule describes the dependence of the radius of curvature remarkably well. The extrapolated value for the radius of curvature for DOPC is approximately 95 Å. The extrapolation is rather sensitive to the parameters used in the fit, but its large value indicates that spontaneously highly curved structures are unlikely to be thermodynamically stable for hydrated DOPC alone. More recently, Keller et al. (1993) have reported a value of R_o = 96 Å for DOPC in the presence of alkane at 23°C by direct measurement, which is in good agreement with the extrapolated value found here.

The values obtained for the headgroup area per molecule, fitted as free parameters in Fig. 6, are A_{PE} = 58 Å^2 and A_{PC} = 78 Å^2, for DOPE and DOPC, respectively. These values are within the range obtained for the corresponding areas per molecule of phosphatidylethanolamines and phosphatidylcholines in the fluid lamellar phase (see, e.g., Marsh, 1990), and correspond to values for the lipid packing parameter of V/Al = 1.08 and 1.34 for DOPC and DOPE, respectively. The former value is consistent with DOPC alone forming hydrated lamellar structures rather than nonlamellar structures. The latter value lies at the lower end of the packing parameters obtained for phosphatidylethanolamines in the absence of added alkane in Fig. 3 and is consistent with DOPE in the fluid state producing inverted hydrated lipid phases, as is observed.

Further evidence for the appropriateness of an addition rule based on the component molecular dimensions is given by the close correspondence between the dimensions of the H_{II} phase of the DOPE/DOPC 3:1 mol/mol mixture and those of dioleoyl monomethyl phosphatidylethanolamine, which has the same average headgroup composition, as pointed out by Gruner et al. (1988). It can be estimated for

*A value of approximately 15 Å was assumed for l by Rand et al. (1990) for the calculation of R_w. Allowing l to be a free parameter in fitting the data of Fig. 6 yields an optimum fit with $l \approx$ 15.5 Å, without markedly changing the optimum values of A_{PE} and A_{PC}. The same holds true if the values of R_w are simultaneously adjusted during the fit so as to correspond to the changes in l (cf. Rand et al., 1990).

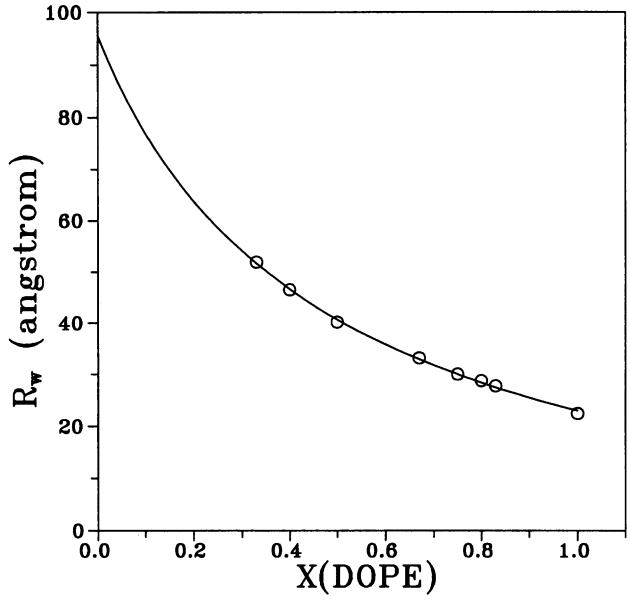

FIGURE 6 Dependence of the radius, R_w, of the aqueous cylinders in the H_{II} phase of DOPC/DOPE mixtures in the presence of excess tetradecane on the mole fraction of DOPE, X(DOPE), at approximately 22°C. The data are taken from Rand et al. (1990). The solid line represents a nonlinear least-squares fit to Eqs. 1, 8, and 9, as described in the text. The parameters fitted are the headgroup area per molecule for DOPE and DOPC.

the latter system that $R_o \approx 30$ Å and $V/Al_{eq} \approx 1.26$ in the presence of dodecane at 20°C.

Energetics of packing in H_{II} and bilayer phases

The curvature energy in an inverted hexagonal phase is given by (see, e.g., Cevc and Marsh, 1987)

$$\Delta G_{curv} = \frac{1}{2}(N_A A k_c)\left(\frac{1}{R_w} - \frac{1}{R_o}\right)^2, \qquad (11)$$

where N_A is Avogadro's number and k_c is the elastic bending modulus. The radius of curvature, R_w, is given by Eq. 3, and the intrinsic radius of curvature, R_o, is given by Eq. 1. This curvature energy can be equated with the energy cost of the additional bending required when the system is under stress due to limiting water content in the phase. In excess water, the H_{II} phase assumes a curvature close to that of the intrinsic curvature, $1/R_o$, hence minimizing the curvature free energy given by Eq. 11 (Rand et al., 1990; Kirk and Gruner, 1985).

For a curved lipid bilayer or membrane, the radius of curvature is equal in magnitude but opposite in sign for the two component monolayers. The value of the curvature that minimizes the total curvature energy for both monolayers is then given by

$$\frac{1}{R_{min}} = \frac{1}{2}\left(\frac{1}{R_{o,i}} - \frac{1}{R_{o,e}}\right), \qquad (12)$$

where $R_{o,i}$ and $R_{o,e}$ are the intrinsic radii of curvature of the "internal" and "external" monolayers, respectively (cf. Fig. 1, A and B, respectively). The minimum curvature energy of the bilayer is then $(N_A A k_c/4)(1/R_{o,i} + 1/R_{o,e})^2$. When the compositions or intrinsic curvatures of the two monolayers are identical, $1/R_{min} = 0$ and a flat lamella has the minimum curvature energy (cf. Introduction). When $R_{o,i} = -R_{o,e}$, the intrinsic curvatures of the two monolayers are exactly complementary and the bilayer tends to a spontaneous curvature $R_o = R_{o,i}$ at which the curvature energy is zero; this is the opposite extreme from the case of a flat bilayer.

The overall scale of the curvature free energy is determined by the bending modulus, k_c. Values of (0.4–2.3) × 10^{-19} J have been measured for phosphatidylcholine bilayers (see Marsh, 1990). The value of most interest here, however, is that for a phospholipid monolayer. The bending modulus is determined by the square of the distance, d_t, separating the two surfaces at which the component forces of the bending moment act ($k_c = \frac{1}{2}K_A \cdot d_t^2$; Evans and Skalak, 1980; Cevc and Marsh, 1987). Therefore, the bending modulus for a monolayer is expected to be a quarter of that for a bilayer, perhaps even less, because the appropriate complementary surface may lie further into the monolayer than the position of the chain ends. It is interesting to note that a more comprehensive analysis of the elastic distortions of H_{II} phases (Rand et al., 1990) has yielded bending elastic constants for the lipid monolayer that are less than half those previously estimated (Kozlov and Winterhalter, 1991). In view of these considerations, even values as low as $k_c \approx 10^{-20}$ J may be appropriate for monolayers (cf. Marsh, 1996). An interesting comparison can be made with the enthalpy of lamellar-inverted hexagonal transitions. Below the transition, the curvature free energy is given by Eq. 11, with $1/R_w = 0$, and above the transition $\Delta G_{curv} \approx 0$, because $R_w \approx R_o$. With curvature elastic constants in the range $k_c = (0.4–2.3) \times 10^{-19}$ J, the net change in curvature free energy at the transition is then $\Delta G_{curv} \approx 1.9–10.7$ kJ.mol^{-1}, for $R_o \approx 19$ Å and $A \approx 56$ Å^2. This range is comparable in magnitude with the hexagonal transition enthalpy for dihexadecyl phosphatidylethanolamine (6.1 kJ.mol^{-1}; Seddon et al., 1983), for which the above values of R_o and A are appropriate (cf. Fig. 3). For the H_{II} phase of DOPE, a monolayer bending modulus of $k_c = (3.8–4.5) \times 10^{-20}$ J (Kozlov and Winterhalter, 1991) has been deduced from the dual-solvent stress data of Rand et al. (1990), where $R_o = 23$ Å and $A = 53$ Å^2. The curvature energy is then estimated to be $\Delta G_{curv} \approx 1.2–1.4$ kJ·mol^{-1}, which may be compared with the hexagonal transition enthalpy of 1.2 ± 0.2 kJ·mol^{-1} (Epand, 1985).

Lamellar-nonlamellar phase transitions

The temperature at which a transition from the lamellar phase to the nonlamellar phase takes place (e.g., the lamellar to H_{II} transition temperature, T_h) is dependent on the curvature elastic energy (Eq. 11). Transition temperature shifts,

e.g., between different lipids, are determined by the differences, $\delta\Delta G_{curv}$, in the elastic energy, according to (see, e.g., Cevc and Marsh, 1987)

$$\Delta T_h = \delta\Delta G_{curv}/\Delta S_h, \tag{13}$$

where ΔS_h is the transition entropy. For lipids with values of intrinsic curvature differing by an amount $\delta(1/R_o^2)$, the shift is given from Eqs. 11 and 13 by

$$\Delta T_h = -\frac{1}{2}\left(\frac{N_A A k_c}{\Delta S_h}\right)\left[\delta\left(\frac{1}{R_o^2}\right) + \frac{1}{R_o^2}\frac{\delta k_c}{k_c}\right], \tag{14}$$

because $R_w \approx R_o$ in the H_{II} phase and $1/R_w = 0$ in the L_α phase. For the moment, any possible difference, δk_c, in the curvature elastic constant will be ignored. From the data given in Fig. 6, the difference in R_o between DOPE and DOPE/DOPC (3/1) is approximately 8 Å, corresponding to a shift in transition temperature of approximately 40°, obtained from Eq. 14 and the value of the calorimetric entropy (Epand, 1985). The experimentally measured difference in L_α-H_{II} transition temperature between the two lipid systems is approximately 50° (Kirk and Gruner, 1985). The theoretical estimate ignores the temperature dependence of R_o and assumes that the contribution from packing constraints in the two systems is comparable. Furthermore, a relatively low value has been assumed for the curvature elastic constant ($k_c \approx 10^{-20}$ J). Similarly, from the data for DOPE and DOPE/DOPC (5.07:1) at 50°C given in Fig. 3, the difference in R_o is 3 Å and the predicted shift in transition temperature is ~26°, compared with ~20–30° measured experimentally (Tate and Gruner, 1989).

The dependence of the transition temperature on molecular dimensions, i.e., on the lipid packing parameter, can be obtained from the resulting changes in R_o predicted from Eq. 1. To first order, the change in the curvature term is given by $\delta(1/R_o^2) = (2/R_o)\delta(1/R_o)$, where

$$\delta\left(\frac{1}{R_o}\right) = \left(\frac{1}{R_o}\right)\left[\frac{\delta(V/Al)}{V/Al - 1} - \frac{\delta l}{l}\right], \tag{15}$$

and $\delta(V/Al)$ is the change in the packing parameter. The dependence of the bending modulus on the molecular dimensions is given by $k_c \propto K_A l^2$ (cf. above), which therefore changes according to $\delta k_c/k_c = +2\delta l/l$. Substituting these results into Eq. 14 then gives the following dependence of the lamellar to inverted hexagonal transition temperature on changes in the lipid packing parameter:

$$\Delta T_h = -\left(\frac{N_A A k_c}{\Delta S_h}\right)\left(\frac{1}{R_o^2}\right)\frac{\delta(V/Al)}{V/Al - 1}. \tag{16}$$

Because $V/Al > 1$ for H_{II} phases, this equation predicts that an increase in packing parameter will decrease the temperature of transition to the inverted hexagonal phase, and correspondingly a decrease in packing parameter will elevate the nonlamellar transition temperature. The former situation (i.e., a decrease in transition temperature) obtains experimentally with increasing chain length and the latter (i.e., an increase in transition temperature) with increasing headgroup size, for phosphatidylethanolamines (Seddon et al., 1983). Presumably, the increase in chain volume dominates the change in packing parameter in the first case and the increase in headgroup area dominates in the second case.

For dialkyl phosphatidylethanolamines, the measured decrease in L_α-H_{II} transition temperature with increasing chain length is $\Delta T_h \approx -4°$ per methylene group, and the transition entropy of dihexadecyl phosphatidylethanolamine is $\Delta S_h = 16.9$ J·mol^{-1}·K^{-1} (Seddon et al., 1983). Taking interpolated dimensions corresponding to the H_{II} phase for a 16-C atom chain length (Seddon et al., 1984) yields a value of $\delta(V/Al)/(V/Al - 1) \approx 0.07$ per chain methylene group from Eq. 16. Taking this value and calorimetric data for dipalmitoyl phosphatidylethanolamine yields a predicted transition temperature shift of $\Delta T_h \approx -22°$ per chain methylene group, compared with a value of $\Delta T_h \approx -21°$ per CH_2 group, which is found experimentally for diacyl phosphatidylethanolamines (Marsh, 1991). For increases in the size of the lipid polar headgroup, the inverted hexagonal transition temperature increases by $\Delta T_h \approx +16°$ per methylene group for phosphatidylalkanolamines, and by $\Delta T_h \approx +24°$ per methyl group for *N*-methyl phosphatidylethanolamines with tetradecyl chains (Seddon et al., 1984). Estimates corresponding to those already given for the chain length dependence yield changes in packing parameter of $\delta(V/Al)/(V/Al - 1) \approx -0.11$ per alkanolamine methylene group and ≈ -0.17 per *N*-methyl group, by using interpolated dimensional values and the transition entropy for ditetradecyl phosphatidylethanolamine. The above estimates for the change in packing parameter have been made by using a value of $k_c \approx 10^{-20}$ J that reproduced the correct orders of magnitude for the difference in transition temperature between DOPE and DOPE/DOPC mixtures. Taking larger values for the curvature elastic constant would yield correspondingly smaller values for the change in packing parameter.

Previous analyses of the shifts in the L_α-H_{II} transition temperatures with molecular structure have been made from estimates of the resulting changes in the components of the lateral pressure in the lipid headgroup and chain regions (Seddon et al., 1983; Marsh, 1986) by using the thermodynamic model of Israelachvili et al. (1976). Here they are related directly to the changes in the molecular geometry by means of the lipid packing parameter and its influence on the curvature elastic energy. The two approaches are complementary and are related by the way in which the lateral pressure components determine overall lipid packing geometry (cf. Marsh, 1996).

CONCLUSIONS

The intrinsic curvature, R_o, of amphiphilic lipid assemblies can be expressed usefully in terms of the lipid molecular packing parameter, V/Al. Use of the equivalent (cylindrical) lipid length, l_{eq}, allows definition of lipid packing parame-

ters for inverted hexagonal phases. These can then be used for interpreting the extensive experimental data available on such phases. Of particular note are the thermal expansion and additive properties in lipid mixtures, as well as transitions to the inverted hexagonal phase. It should be emphasized that operationally the packing parameter is a generalized quantity that effectively includes interactions between the lipid polar headgroups and between the lipid hydrocarbon chains. Therefore strictly it can only be determined experimentally from measurements on systems of known curvature, as has been illustrated here.

APPENDIX

Geometrical considerations

Expressions for the radius of curvature in terms of the packing parameter are obtained from geometrical results for the volume-to-surface ratios, *V/A*, for the various structures (see Fig. 1).

For a cylindrical tube of length L that contains n lipids, the area per molecule at the relevant surface is given by

$$A = 2\pi RL/n \tag{A.1}$$

for both normal and inverted structures (Fig. 1, *A* and *B*). For an inverted cylindrical structure (Fig. 1 *A*), the volume per lipid is given by

$$V = \pi[(R + l)^2 - R^2]L/n, \tag{A.2}$$

and for a normal cylindrical structure (Fig. 1 *B*):

$$V = \pi[R^2 - (R - l)^2]L/n, \tag{A.3}$$

where the radii of curvature are defined in the corresponding figures. The solution for R is linear and, for both cases, can be written in the form of Eq. 1, with the sign convention for R given in the text, viz. positive for inverted and negative for normal structures, respectively.

For a spherical shell of n lipids, the area per molecule at the polar surface is given correspondingly by

$$A = 4\pi R^2/n. \tag{A.4}$$

For inverted spherical structures (Fig. 1 *A*) the volume per lipid is given by

$$V = (4\pi/3)[(R + l)^3 - R^3]/n, \tag{A.5}$$

and for normal spherical structures (Fig. 1 *B*)

$$V = (4\pi/3)[R^3 - (R - l)^3]/n. \tag{A.6}$$

A quadratic solution is obtained for R, for which the positive sign of the square root is appropriate. This is given by Eq. 2 with the sign convention for normal and inverted structures that was already mentioned.

For a H_{II}-phase hexagonal tube of length L that contains n lipids, the area per lipid at the polar surface is given by

$$A = 2\pi RL/n. \tag{A.7}$$

The volume per lipid in the hexagonal tube is given by (cf. Fig. 1 *C*)

$$V = [3(l_{max} + R)^2\sqrt{3}/2 - \pi R^2]L/n \tag{A.8}$$

or, alternatively, by

$$V = [3(l_{min} + R)^2 2/\sqrt{3} - \pi R^2]L/n. \tag{A.9}$$

A quadratic solution is obtained for R, for which the negative sign of the square root is appropriate (cf. Tate and Gruner, 1989). This is given by Eq. 3, where the packing parameter is defined in terms of either l_{max} or l_{min}.

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