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Letter to the Editor

Nonlamellar Packing Parameters for Diacylglycerols

In a recent paper in this journal, Leikin et al. (1996) obtained values for the dimensions of the inverted hexagonal (H_{II}) phases of dioleoyl phosphatidylethanolamine (DOPE)/dioleoyl glycerol (DOG) mixtures at full hydration and under various conditions of solvent stress by using x-ray diffraction. It was demonstrated that a unique pivotal plane exists in the lipid H_{II} structures for which the molecular area is constant, independent of both water stress and lipid composition. Most interestingly, it was found that the spontaneous curvature ($1/R_{op}$) of the pivotal surface was a linear function of the mole fraction of the DOG component in the mixtures. Recently I considered the dimensions of H_{II} phases in terms of a simple geometric lipid packing parameter (Marsh, 1996). The packing parameter was defined as V/Al_{eq}, where V is the lipid molecular volume, A is the molecular area at the lipid-water interface, and l_{eq} is the equivalent lipid length for a cylindrical shell with lipid volume equal to that of the hexagonal prisms in the H_{II} phase. The latter is given by $l_{eq} = \sqrt{2\sqrt{3}/\pi}(l_{min} + R_w) - R_w$, where l_{min} is the minimum lipid length (i.e., the length along the intercylinder axis) in the H_{II} phase, and R_w is the radius of the water cylinders. A straightforward geometrical result is then obtained for the curvature of the Luzzati/Gibbs dividing surface:

$$1/R_w = 2(V/Al_{eq} - 1)/l_{eq} \tag{1}$$

i.e., of the water cylinders in the H_{II} phase. An addition rule was proposed for the spontaneous surface curvature based on linear additivity for V and A according to mole fraction of the component lipids, which corresponds to conservation of molecular volume and to preservation of headgroup packing density *at maximum hydration*. The latter differs from the situation at the pivotal plane, where the molecular area is also insensitive to the degree of hydration. In contrast, it is known that the area per lipid headgroup in general depends on water content (e.g., Marsh, 1990).

It is of considerable interest to see whether the addition rule proposed for fully hydrated H_{II} phases and found to hold for DOPE/dioleoyl phosphatidylcholine (DOPC) mixtures (Marsh, 1996) also applies for mixtures containing the second-messenger lipid diacylglycerol. The compositional dependence of the radius of the water cylinders in the H_{II} phase of DOPE/DOG mixtures is given in Fig. 1. From the data of Leikin et al. (1996) it is found that the equivalent length of a lipid molecule remains constant at a value of $l_{eq} = 17.5$ Å, independent of the DOG content, for the fully hydrated mixtures. The dependence of R_w on X_{DOG} can then be fit well by Eq. 1, with molecular volumes $V_{DOPE} = 1235$ Å^3 and $V_{DOG} = 1116$ Å^3 (Leikin et al., 1996), taking the molecular areas at the lipid-water interface, A_{DOPE} and A_{DOG}, as free parameters, and assuming linear additivity according to the mole fraction, X_{DOG}, for both V and A (see Fig. 1). This shows that the molecular areas at the lipid-water interface remain approximately constant, independent of DOG content, and yields fitted values of $A_{DOPE} = 50.0 \pm 0.1$ Å^2 and $A_{DOG} = 17.2 \pm 0.6$ Å^2, with $\chi^2 = 0.03$ Å^4. The corresponding values of the lipid packing parameters are $(V/Al_{eq})_{DOPE} = 1.41$ and $(V/Al_{eq})_{DOG} = 3.70$ for DOPE and DOG, respectively. The latter corresponds to a notional water radius of the H_{II} phase for DOG of $R_w = 3.2$ Å, the small value of which is consistent with the fact that DOG alone does not form H_{II} phases but only isotropic dispersions of nonhydrated oil droplets in the fluid phase (Schorn and Marsh, 1996).

The values of the molecular area and packing parameter for DOPE obtained here differ from those found previously (Marsh, 1996) from a similar analysis of data on DOPE/DOPC mixtures in the presence of excess tetradecane obtained by Rand et al. (1990). For the latter, it was assumed that the appropriate value of the lipid length, l, in Eq. 1 was that along the intercylinder axis (i.e., $l_{min} \approx 15.5$ Å) because the packing restraints on the H_{II} cylinders are relieved by the tetradecane (see Marsh, 1996). If a value of $l = 17.5$ Å (i.e., equal to l_{eq} for the DOPE/DOG mixtures) is assumed for the DOPE/DOPC mixtures, then the fitted values obtained for the molecular areas are $A_{DOPE} = 49.9 \pm 0.1$ Å^2, $A_{DOPC} = 68.3 \pm 0.1$ Å^2 ($\chi^2 = 0.15$ Å^4), corresponding to packing parameters $(V/Al)_{DOPE} = 1.38$ and $(V/Al)_{DOPC} = 1.09$. For DOPE, these values are then close to those obtained here with the DOPE/DOG mixtures in the absence of tetradecane.

The addition rule proposed for determining the curvature of the Luzzati/Gibbs dividing surface may therefore be applied usefully to determine the radius of the water cylinders in lipid H_{II} phases containing diacylglycerol. A precondition for this is that the headgroup packing density at maximum hydration should be essentially independent of lipid composition. To a first approximation, this is implicit in the measurements of the molecular area, A_w, at the lipid-water interface of the DOPE/DOG mixtures over the range studied (Leikin et al., 1996; see *inset* to Fig. 1), although a small systematic nonlinear trend cannot be excluded. The linear regression given in the inset to Fig. 1 yields values of $A_{DOPE} = 49.9 \pm 0.2$ Å^2 and $A_{DOG} = 16.6 \pm 1.2$ Å^2 ($r = 0.996$), which are consistent with the corresponding values obtained by fitting the dependence of R_w on lipid composition. The approximate constancy of the molecular areas, in turn, is consistent with the thermody-

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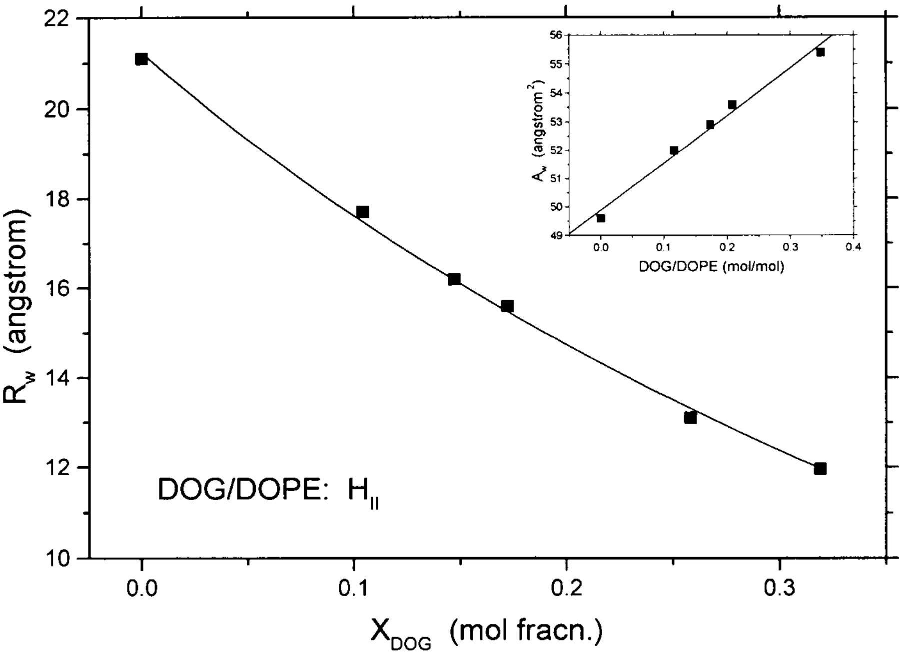

FIGURE 1 Dependence of the radius, R_w, of the aqueous cylinders in maximally hydrated H_{II} phases of DOPE/DOG mixtures on the mole fraction of DOG, X_{DOG}. The data are taken from Leikin et al. (1996). The solid line represents a nonlinear least-squares fit to Eq. 1, with $V(\text{Å}^3) = 1235(1 - X_{DOG}) + 1116X_{DOG}$, $A = (1 - X_{DOG})A_{DOPE} + X_{DOG}A_{DOG}$, and $l_{eq} = 17.5$ Å. (The rightmost point is back-calculated from data given in the original paper and does not appreciably change the fit.) (*Inset*) Dependence of total lipid/water area per DOPE molecule, A_w, on DOG/DOPE molar ratio (data from Leikin et al., 1996).

namic concept of an optimum equilibrium interfacial area/lipid molecule in unstressed lipid/water systems (e.g., Israelachvili et al., 1976), although this may be modulated somewhat by the particular chain packing constraints present in H_{II} phases.

It will be noted that the position of the pivotal plane, which is defined as the dividing surface for which the molecular areas remain rigorously constant, does not coincide with the Luzzati/Gibbs dividing surface, i.e., with the lipid-water interface (Leikin et al., 1996). This does not imply, however, that the lipid headgroup packing density at maximum hydration may not remain approximately constant, within experimental accuracy, at the lipid-water interface. This is especially the case when one considers that it is the fractional lipid *volume* between these two surfaces that remains constant, which does not exclude the possibility that their radial separation may vary. (From the data given below, it can be estimated that the latter increases from the region of 6.9 Å to 7.4 Å with increasing DOG content.)

In the present instance, the near-constancy of the molecular areas at the lipid-water interface can be further illustrated by the diagnostic plot of $(A_w/V_l)^2$ versus $A_w/(V_lR_w)$, which was used by Leikin et al. (1996) to determine the position of the pivotal surface. Here V_l is the volume of an effective DOPE + $X_{DOG}/(1 - X_{DOG})$ DOG molecule. Synthetic data were calculated for lipid compositions corresponding to those employed in the experiment by using Eq. 1. The additivity of molecular areas was assumed for A_w and that of molecular volumes was assumed for V_l, with the component values equal to those that were used for the nonlinear fit in Fig. 1. Linear regression then yields values of $A_p = 0.0537$ Å$^{-1} \times V_l$ for the area of the effective molecule at the pivotal plane and $V_p = (0.328 \pm 0.005)V_l$ for the volume between the pivotal and Luzzati/Gibbs planes ($r = -0.9997$, $N = 5$). These may be compared with the corresponding values deduced from the experimental data for DOPE/DOG mixtures at maximum hydration and with the same compositions: $A_p = 0.0531$ Å$^{-1} \times V_l$ and $V_p = (0.318 \pm 0.002)V_l$ ($r = 0.9999$, $N = 5$). The near-linearity of the diagnostic plot generated assuming exact additivity of the molecular areas at the lipid-water interface and the near-agreement of the regression parameters with those obtained from direct experiment again support the assumption of approximately constant molecular areas.

In principle, Eq. 1 (being purely geometric) should apply to any choice of dividing surface. For the pivotal plane of DOPE/DOG mixtures, it was found that the corresponding V/A ratio ($= (V_l - V_p)/A_p$) was constant, being independent of DOG (and water) content (Leikin et al., 1996). The finding that the spontaneous curvature, $1/R_{op}$, is linearly dependent on X_{DOG} is therefore not a simple geometric result. Rather, it reflects some deeper feature of the molecular interactions that controls the way in which the position of the pivotal plane determines the corresponding values of l_{eq} for the portion of the lipid that lies outside this plane (cf. the two previous paragraphs).

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