THE ADSORPTION OF NONPOLAR MOLECULES INTO LIPID BILAYER MEMBRANES

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ABSTRACT Thermodynamic considerations show that the adsorption of nonpolar molecules into lipid bilayer membranes should depend upon the curvature of the membranes. Estimations of the difference in adsorption of a small *n*-alkane between a planar phospholipid bilayer and liposomal vesicles have been attempted. For spherical multilamellar liposomes exposed to saturated solutions of alkane in water the adsorption is calculated to be 17–65% of the value for the planar bilayer, depending on the assumptions in the model.

Several recent papers have been concerned with the adsorption of nonpolar molecules, particularly hydrocarbons, into lipid bilayers (1–7). The results which have been reported were obtained with two quite different types of system, planar bilayers or black lipid films on the one hand, and suspensions of multilamellar liposomes on the other. Where comparison is possible, it appears that adsorption into the bilayers of the multilamellar structures is substantially smaller than into the planar bilayer. The reasons for this discrepancy are of some importance since both types of system are used as indicators of the adsorption to be expected into the lipid regions of biological membranes. The purpose of this paper is to draw attention to the fact that, in general, adsorption into the two systems should not be similar and to show that, owing to their curved surfaces and multilamellar structure, adsorption into liposomes could be considerably smaller than into single planar leaflets.

At a qualitative level, the general features of the adsorption of alkanes into lipid bilayers are fairly well established. It is known that, in black lipid films of egg yolk lecithin the area per molecule of the lipid is, to within experimental error, the same for films formed from *n*-decane or from *n*-hexadecane (1) and again that, to within experimental error, this area per molecule is similar to that obtained from X-ray studies of liquid crystalline phospholipid in excess aqueous solutions (19, 20, 24). Parallel studies of black films of glyceryl monooleate also show that the area per molecule of the lipid is independent of the alkane used and the extent to which it is adsorbed (9, 16, 21). It follows that the bilayer must become thicker on adsorption of alkane, a conclusion supported by electrical capacity and light reflectance measurements (1–3, 9, 10). Furthermore, since at an interface between water and a solution or dispersion of a lipid in hydrocarbon the lipid is adsorbed very strongly relative to the hydrocarbon, the Gibbs surface excess of the latter substance relative to the former is negative. In such circumstances, the Gibbs equation requires that an increase in the hydrocarbon concentration (strictly, activity), or dilution of the lipid, results in an increase in the interfacial tension. We should expect, therefore, that adsorption of hydrocarbon into a bilayer would lead to an increase in its tension and, from black film experiments, this appears to be so (2, 3).

The chemical potential, μ_i^σ, of a hydrocarbon (i) in a lipid bilayer may be written (11, 12)

$$\mu_i^\sigma = \mu_i^{+,\sigma}(p_0) + (p - p_0)\overline{V}_i^\sigma + RTlnF(x_i^\sigma) - \sigma\overline{a}_i. \tag{1}$$

$p - p_0$ Is the pressure in excess of atmospheric which acts on the bilayer; $\overline{V}_i^\sigma$ is the partial molar volume of i; $F(x_i^\sigma)$ is a function of x_i^σ, the mole fraction of i, which formally includes the activity coefficient of i in the bilayer; σ is the bilayer tension (defined as $(\partial A^\sigma/\partial \mathcal{A})_{T,V^\sigma,n_i^\sigma,n_j^\sigma}$, where A^σ is the Helmholtz free energy and $\mathcal{A}$ is the area of the bilayer); $\overline{a}_i$ is the partial molar area of i in the bilayer (defined as $(\partial \mathcal{A}/\partial n_i^\sigma)_{T,V^\sigma,n_j^\sigma,\sigma}$). When i in the bilayer is in equilibrium with i at a given chemical potential in the surrounding aqueous media, $F(x_i^\sigma)$ is a function of $(p - p_o)\overline{V}_i$ and $\sigma\overline{a}_i$.

As a model for calculation we have assumed that adsorption of a *n*-alkane occurs into a suspension, in an aqueous solution, of spherical multilayered liposomes of a phosphatidylcholine (See Fig. 1). The individual bilayers will tend to thicken, their tensions will tend to increase and, because their surfaces are curved, a pressure differential will develop across them. This pressure will act on the aqueous as well as the hydrocarbon layers and thus both these spacings are likely to change. If r_i is the radius of the i^{th} concentric leaflet and ℓ is the repeat distance (taken as 62.1 Å [13] in the initial spherule of pure lipid, the radius r_{i+1} of the $(i+1)^{th}$ bilayer will be given by

$$r_{i+1} = r_i + \ell + \Delta h_i^\omega + (\Delta h_i^\ell + \Delta h_{i+1}^\ell)/2. \tag{2}$$

Δh_i^ω Is the (algebraic) thickness change in the i^{th} water layer after adsorption of alkane and Δh_i^ℓ and Δh_{i+1}^ℓ are the corresponding values for the i^{th} and $(i + 1)^{th}$ lipid layers; r_{i-0} is assumed to be 100 Å.

We define the pressure difference Δp_i^ℓ as the pressure in excess of atmospheric which acts

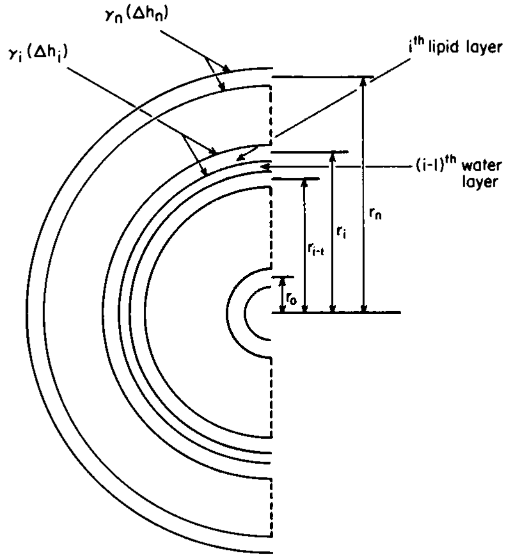

FIGURE 1 A representation of a spherical, multilamellar droplet of the liquid crystalline phase of a phospholipid, showing the assignment of the parameters used in Eqs. 2–5.

within the i^{th} lipid layer. A corresponding term Δp_i^{ω} is defined for the i^{th} water layer. These pressures are assumed to be given by forms of the Laplace equation. Thus,

$$\Delta p_i^{\ell} = \frac{2\gamma_i(\Delta h_i)}{r_i} + \sum_{j=i+1}^{j=n} \frac{4\gamma_j(\Delta h_j)}{r_j} \tag{3}$$

and

$$\Delta p_i^{\omega} = \sum_{j=i+1}^{j=n} \frac{4\gamma_j(\Delta h_j)}{r_j}, \tag{4}$$

where $\gamma_i(\Delta h_i)$ and $\gamma_j(\Delta h_j)$ are the tensions of the surfaces of the i^{th} and j^{th} leaflets respectively (i.e., $\sigma_i/2$ and $\sigma_j/2$) and there are a total of $n + 1$ bilayers. Thus, the Δp's depend upon the summed pressure drops across all other bilayers closer to the liposome surface. The tensions, which depend on the amount of alkane adsorbed, will be assumed to be similar to those of black phospholipid films containing the same amount of alkane. This assumption neglects the van der Waals and electrostatic interaction forces which cause the bilayers to aggregate into a multilamellar structure. However, in the present systems these interactions are thought to be small and to act in the same direction as Δp_i^{ℓ}. Estimates of γ_i may be obtained by considering the interfacial tensions measured for black films formed from alkanes of various chain length and hence which contain different volume fractions of hydrocarbon (Fig. 2). The most comprehensive and reliable set of results is for glyceryl monooleate films, where it can be seen that there is a roughly linear relationship between the tension and the adsorption. For dioleylphosphatidylcholine in the pure state it is widely recognized that the tension is very small, and is here assumed to be zero. For the same lipid (or for egg phosphatidylcholine), black films formed from decane have interfacial tensions of 1–3 dyn cm^{-1} (3, 14) and it is assumed that, as for the glyceryl monooleate, there is a roughly linear dependence of tension

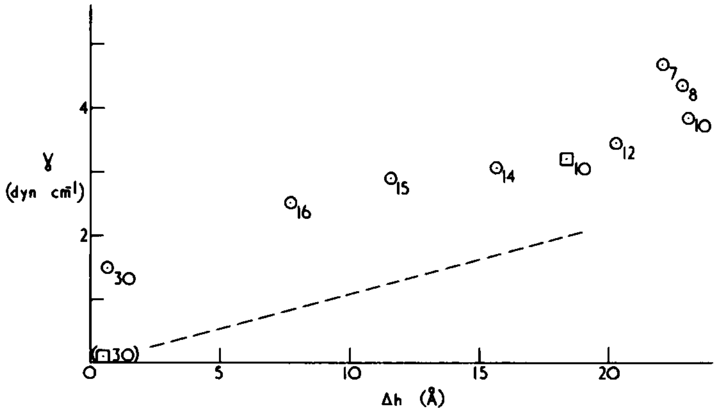

FIGURE 2 Interfacial tensions (γ) of black lipid films as a function of alkane adsorption. The thickening (Δh) is proportional to the volume adsorbed. ⊙, glyceryl monooleate films; ⊡, dioleyl phosphatidylcholine films. The numbers beside the points indicate the number of carbon atoms in the alkane. The dashed line is the relationship assumed (Eq. 5) for present purposes. (Data from references 1–3 and J. R. Elliott, personal communication.)

on adsorption. Thus, for purposes of calculation we have used the expression

$$\gamma_i = 1.064 \times 10^7 \times \Delta h_i^\ell, \tag{5}$$

where Δh_i^ℓ is in centimeters and γ_i is in dyn cm^{-1}. The constant has been deduced from the slope of the dashed line in Fig. 2.

The pressure Δp_i^ℓ which tends to develop within each bilayer opposes the adsorption of the alkane. This is formally expressed by a set of equations similar to Eq. 1, one for each layer, where at adsorption equilibrium, μ_i would be the same throughout the multilayered structure.

However, to evaluate the adsorption of alkane into the multilayered structure we require an explicit expression for x_i^σ as a function of $(p - p_0)\overline{V}_i^\sigma$, $\sigma\bar{a}_i$ and the curvature of the bilayer. Such an expression is not available and to obtain quantitative results rather crude empirical methods have to be employed.

Electrostrictive pressure applied to a black film can significantly reduce its thickness through squeezing out predominantly the alkane solvent into a reservoir of nearly pure liquid alkane at effectively atmospheric pressure (15–18). It is assumed that the area per molecule of the lipid remains almost constant during this process and, hence, an expression linking excess pressure to the adsorption of alkane can be derived. Two empirical expressions have been fitted to the experimental results for a glyceryl monooleate-decane black film (Fig. 3). The first of these may be written

$$\Delta p_i^\ell = 10^4[e^{1.83 \times 10^{14}(2.622 \times 10^{-7} - \Delta h_i^\ell)^2} - 3.06], \tag{6a}$$

where Δp_i^ℓ is expressed in dyn cm^{-2} and Δh_i^ℓ in centimeters. This equation follows from that given previously (2) if the van der Waals forces are taken as constant and equal to their value for a film of thickness 48 Å. This simplification does not significantly affect the results but greatly facilitates the calculation. The second expression is

$$\Delta p_i^\ell = 9{,}827\,(18.4 - 10^8 \Delta h_i^\ell)^{1.591}, \tag{6b}$$

where the units are as in Eq. 6a. While both Eqs. 6a and 6b fit the experimental results well they diverge enormously for smaller values of Δh_i^ℓ (Fig. 3). Since the experimental results cover only about one-third of the thickness range of interest it is not at all certain which curve is physically the more realistic. Nevertheless, since the equations, between them, cover a wide range of behavior it is likely that the actual system would have intermediate properties. (It may be noted that neither equation embodies the requirement that $\Delta p_i^\ell \longrightarrow \infty$ as $\Delta h_i^\ell \longrightarrow 0$ but that this will not materially affect our main conclusions.) One further consideration is that the results of Fig. 3 are for a monoglyceride whereas the liposome envisaged is of phospholipid. Although there are no comparably detailed studies of the phospholipids, it is well established that their response to applied pressures is similar to that of glyceryl monooleate (15, 17).

The dependence of the thickness of the water layers on the pressure Δp_i^ω has been ascertained experimentally (13). Empirical relationships which describe these findings are

$$\Delta h_i^\omega = 0 \qquad (\Delta p_i^\omega \lesssim 10^5 \text{ dyn cm}^{-2}) \tag{7}$$

$$\Delta h_i^\omega = (17.19 - 3.45 \log_{10} \Delta p_i^\omega)\, 10^{-8}\ (10^8 \gtrsim \Delta p_i^\omega \gtrsim 10^5 \text{ dyn cm}^{-2}), \tag{8}$$

where the units are as in Eq. 6.

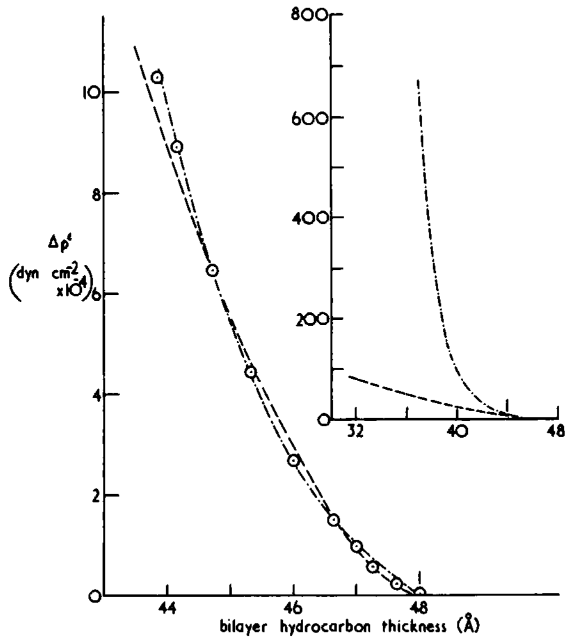

FIGURE 3 The pressure-thickness relationship for a black film of glyceryl monooleate and *n*-decane. The curves ----- and ---, corresponding to Eqs. 6a and 6b respectively, are shown in relation to the points obtained from experiment, ⊙, (2). In the inset, where the units are the same, the behavior of the two expressions is shown over the pressure range of interest in the present calculations.

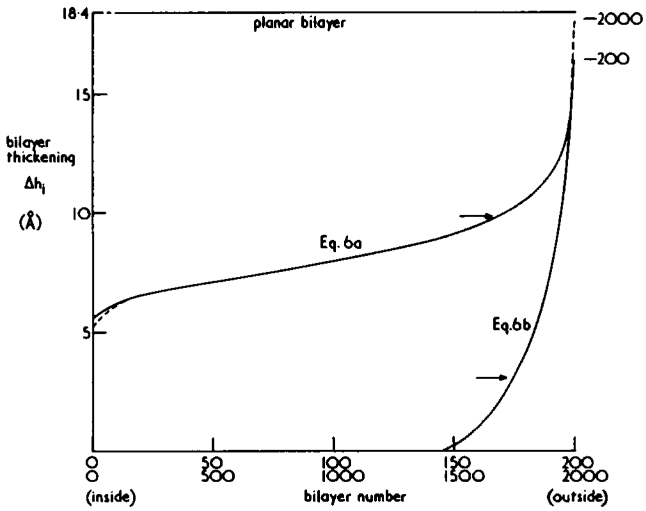

FIGURE 4 The predicted thickening (which is proportional to the volume of alkane adsorbed per unit area of membrane) of the various bilayers of 200- (continuous) and 2,000-layer (dashed curves) spherical liposomes in an aqueous solution saturated with a small alkane, e.g., *n*-hexane. The upper and lower curves were derived using Eqs. 6a and 6b, respectively. The average thickening is indicated by the arrows. The horizontal line at the top of the diagram is the thickening for a planar bilayer.

Eqs. 2–8 have been solved iteratively on a computer. The results are shown in Fig. 4 and correspond to what should occur in an aqueous solution saturated with, say, *n*-hexane or *n*-decane. When Eq. 6a is used and 2,000 bilayers are assumed in the liposome, the upper curve (with dashed variants) is obtained. As can be seen the outermost bilayer adsorbs almost as much alkane as the planar bilayer (represented by the horizontal line at 18.4 Å). However, the adsorption or thickening falls rapidly over the first hundred bilayers, then more slowly, reaching 5 Å in the center. The average alkane adsorption per mole lipid, which is determined mainly by effects in the outer part of the structure, is found to be 54% of the value for the planar bilayer. The smaller 200-layer liposome, apart from a lower adsorption in the outer layers gives a very similar pattern and the average adsorption is again 54% of the planar value. For a single-walled vesicle of radius 100 Å, where the aqueous core is assumed incompressible, the equations predict that the alkane adsorption would be 50% of the planar value.

Eq. 6b represents a much greater sensitivity of adsorption to pressure and yields the lower curve in Fig. 4 for a 200-layer liposome. Fifty layers in from the outside the adsorption is almost zero, and the average adsorption per mole lipid is only 17% of the planar value. For a single-walled vesicle, 19% is found in contrast to the 50% given above.

To test the sensitivity of the results to the dependence of bilayer tension on alkane adsorption calculations were repeated, using Eq. 6a, but in which the slope of Eq. 5 (Fig. 2) was made first twice and then one-quarter of its nominal value. The average adsorptions were 48 and 65%, respectively, of the planar value.

How is the elevated pressure at the center of the liposome supported? By an argument similar to that of Tanford (22) if the water in the centre has bulk properties then, at equilibrium, it must be at the same pressure as the water outside the liposome. If this is so, then the innermost lipid layers must support all the pressure built up by the bilayers closer to the liposome surface. For a bilayer with a radius of curvature comparable to its thickness there are obvious ways (discussed briefly by Tanford [22]) in which adequate counter stresses can be set up. It is also possible that impermeable solutes trapped in the liposome or an electrical diffuse layer may contribute appreciable osmotic pressures as the central cavity tends to shrink. Nevertheless, the results predicted for the innermost layers by the above treatment may be quite inaccurate. Whether or not this is so is, however, not important for the present thesis since the average adsorption is very insensitive to the behavior of the innermost layers.

Two experimental results may be compared with the above predictions. Simon et al. (4) report a value for *n*-hexane at saturation which corresponds to roughly 5%, while Miller et al. (6) find for *n*-butane at 1 atm ~30% of the planar value. The latter result is not easy to compare quantitatively with the results for liquid alkanes but is certainly in the range expected theoretically. The former value is lower than our most extreme predictions.

It is possible to argue that because the black film or planar bilayer has a border the adsorption into this structure may not be comparable, even at similar pressures, with that for a leaflet of a liposome. For at least two reasons the authors believe that the adsorption should be comparable. First, it has been shown that when the solvent content of a phospholipid black film is reduced to below a certain level (e.g., as in an egg lecithin-hexadecane film [1]) it expands spontaneously, suggesting that its tension is virtually zero. In such a black film, the physical state of the lipid molecules must be almost identical to their state in a large single walled vesicle. The fact that the near solventless black film eventually breaks away from its support and reverts (presumably) to a multilamellar liposomal structure is explained by the presence of attractive forces between the bilayers of the liposome which are absent in the black film. When this observation is taken together with the apparent similarity in thickness and area per molecule of the near solventless planar bilayer, and the liquid crystalline leaflet, it seems that only the presence of the solvent produces any significant differences between the two structures. Secondly, although the planar structure has a support, which, as White et al. have argued (23), implies the presence of a thick border even in the so-called solventless case, this border merely balances the tension of the bilayer in the same way as does the excess pressure in the liposomal structure; the capillary pressure exerted by the border on the planar bilayer is negligibly small for present purposes. The border does, however, provide a reservoir of lipid which affects the energetics of area changes. Thus, while increases in area of a planar bilayer may take place at constant area per lipid molecule, the swelling (due to adsorption) of a liposome must stretch the surfaces and increase the area per lipid molecule. Since only hydrocarbon can occupy the extra space, there would be an additional tension increase and thus, an additional constraint to adsorption. Our calculations suggest that this effect is small and, for this reason, it has not been included in the above estimations.

Most biological membranes have average radii of curvature sufficiently large that the pressure effects mentioned are minimal. Their adsorption of nonpolar molecules should therefore correspond closely to that for planar bilayers with comparable compositions.

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