The lipid bilayer as a 'solvent' for small hydrophobic molecules

The cell membrane is viewed at present as a two-dimensional solution in which the lipid bilayer acts as a viscous 'solvent' for oriented integral proteins. Accepting this model as a working hypothesis, questions arise as to the nature of the hydrophobic interior of the bilayer and what rules proteins, lipoproteins, or polypeptides obey in interacting with it. The simplest hypothesis for the interior is that it is equivalent to a bulk alkyl solvent (albeit a very thin one). If this hypothesis is correct, the enthalpy of transfer ($\Delta H$) of a solute molecule (for example, an alkane) from a bulk alkyl solvent to the bilayer interior should be small. That is, the solute–solvent interaction energy should be about the same in the bilayer as in the bulk alkyl. I have examined this hypothesis experimentally by measuring the solubility of n-hexadecane in planar bilayer membranes formed from glycerol-1-monolaurate (1-GMO). The acyl chain of 1-GMO is approximately equivalent to 1-heptadecane. The enthalpy of transfer of n-hexadecane from pure liquid into 1-heptadecane can be estimated from solubility parameter theory to be only a few calories per mol. Thus, the mixing of n-hexadecane in the bilayer interior should be nearly athermal: I have found, however, that $\Delta T$ is orders of magnitude larger than expected. This result has important implications for understanding the bilayer as a two-dimensional solution.

The thickness, and therefore the specific electrical capacitance of, planar bilayer membranes depends strongly on temperature, because the bilayer concentration of alkane solvent (used to form the bilayer) is temperature dependent. The bilayer concentration is believed to be in physicochemical equilibrium with the bulk Plateau–Gibbs border (annulus) surrounding it and numerous micelles of excess solvent trapped within it. Consequently, the planar bilayer may be viewed as a bilayer solution at saturated equilibrium with bulk alkane. Therefore, if the composition of the bilayer is measured as a function of temperature, the thermodynamics of transfer of alkane from the bulk state (micelles and annulus) to the bilayer interior can be estimated using standard equations for saturated solutions. In this way, the interior of the bilayer can be compared directly with the interior of the bulk alkane solvent.

I estimated bilayer composition at various temperatures from measurements of specific geometric capacitance ($C_g$) using methods described in detail elsewhere (see Fig. 2 for summary), and the results at equilibrium conditions are shown in Fig. 1. The points can accurately be fitted with a straight line ($r = -0.9987$). The mole fraction ($X_b$) of n-hexadecane was calculated for each point and $\ln X_b$ plotted against $1/T$ and $\ln T$ (Fig. 2a and b). The enthalpy ($\Delta H$) and entropy ($\Delta S$) of transfer of hexadecane from bulk to bilayer were calculated from these plots and found to be $+3.79 \pm 0.10$ kcal mol$^{-1}$ and $+12.6 \pm 0.3$ entropy units (EU) respectively. These large values of $\Delta H$ and $\Delta S$ indicate that the n-hexadecane molecules are not bound as tightly in the bilayer as in bulk and that they have more motional freedom. One explanation is that, in general, the cohesive forces in the bilayer are not as large as in bulk. The work of Gershfeld and Pagano and Katz indicates that the cohesive forces between acyl chains in monolayers and bilayers are the same as in bulk. An alternative explanation is that the hexadecane molecules are located largely in the deep interior of the bilayer between the monolayers. Numerous X-ray diffraction studies of bilayers reveal a zone of low electron density in the bilayer mid-plane due to the methyl ($-\text{CH}_3$) groups (see, for example, ref. 18). There is thus little interdigitation between the apposed mono-

![Fig. 1 Temperature dependence of the specific geometric capacitance ($C_g$) of planar bilayers formed from glycerol-1-monolaurate (1-GMO). Aqueous phase; unbuffered 0.1 M NaCl. The curve through the points is a least squares fit to $C_g = a + bT$ where $a = 0.7005 \pm 0.0026$ (s.d.) $\mu F \text{cm}^{-2}$ and $b = -0.00471 \pm 0.000079$ (s.d.) $\mu F \text{cm}^{-2} \text{K}^{-1}$. Each point is the mean of measurements on three membranes. (error bars or point diameter, s.e.m.). $C_0$ of each membrane was determined in triplicate. Desorption measurements at the air–water interface using a Langmuir balance indicate that the 1-GMO is slightly soluble in the aqueous phase and that the solubility increases as temperature decreases (S. H. W. and N. L. Gershfeld, unpublished). The aqueous phase was therefore equilibrated at 20°C with the bulk lipid solution (10 mg GMO per ml). Each membrane was allowed to 'age' for 10–20 min to ensure equilibration between micelles and bilayer. In these conditions, $C_0$ was invariant in time within the limit of $\pm 0.3\%$, and I believe the membranes were in physicochemical equilibrium.

![Fig. 2 Temperature dependence of the composition of GMO-hexadecane membranes in a plot of $\ln X_b$ against $1/T$ and $b$, $\ln X_b$ against $\ln T$, where $X_b$ is the mole fraction of n-hexadecane in the bilayer. $X_b$ was estimated from the values of $C_g$ using standard techniques. Briefly, bilayer thickness $\delta_b$ was calculated from $C_g = \varepsilon_r \varepsilon_0 \delta_b$ where $\varepsilon_r = 8.85 \times 10^{-14}$ F cm$^{-2}$ and $\varepsilon_0$ is the dielectric constant of the hexadecane. $X_b$ can be calculated straightforwardly using the bulk densities and dielectric coefficients of the acyl chains (AC) (ref. 17) and hexadecane solvent (S), the number $N_{ac}$ of acyl chains per unit area of bilayer, and the equations $\delta_b = \sqrt{\varepsilon_r \varepsilon_0 N_{ac}}$ and $X_b = \mu_b (N_{ac} + N_{ac})^{-1}$; $\mu_b$ represents the bulk mole fraction of bilayers, and Pagano et al. have reported values for $N_{ac}$ at single temperatures; estimated values at other temperatures using correction procedures similar to those described elsewhere. The corrections are slight and the errors incurred without them are small. At 20°C, $N_{ac} = 5.26 \times 10^{-4}$ cm$^{-2}$ (ref. 10) and $X_b = 0.205$. The curve is a linear regression ($r = 0.9989$) whose slope ($-R_t^2 \ln X_b / \ln T$) gives the enthalpy ($\Delta H$) of transfer of n-hexadecane from bulk to bilayer. $\Delta H = +3.79 \pm 0.10$ kcal mol$^{-1}$, $b$, Also a linear regression ($r = 0.9989$) whose slope ($R_t \ln X_b / \ln T$) gives the entropy ($\Delta S$) of transfer of n-hexadecane from bulk to bilayer. $\Delta S = +12.6 \pm 0.3$ EU.}
layers and a well defined interface must exist between the monolayers. Because the methyl groups have a lower polarisability than of (–CH₂–)₂ groups, molecules located chiefly in the mid-plane should be subjected to smaller van der Waals’ cohesive forces than if they are located parallel to methylene-rich acyl chains within the monolayers. Some hexadecanes must, of course be situated within the monolayers, but simple calculations show that these probably account for ≲ 20% of the total.

I therefore propose that the mid-plane of the bilayer is a special region because of the high concentration of methyl groups with smaller cohesive forces than found between the methylenes of the acyl chains. Support for this hypothesis is given by Dean and Hayes¹⁰ who measured the heat of sorption of hexane vapour on close-packed stearic acid monolayers at the vapour–water interface. At low partial pressures of hexane (that is, low surface coverage by adsorbed hexanes), the heat of sorption is 4.5 kcal mol⁻¹. At high partial pressures (high surface coverage approaching closest packing of hexanes on the stearic acid surface) the heat of sorption becomes identical to the heat of vaporisation of liquid hexane (7.5 kcal mol⁻¹). This indicates that the exposed –CH₃ surface of the stearic acid does not attract the hexanes as strongly as the fully covered hexane surface or bulk liquid.

Experiments are in progress to examine the thermodynamics of transfer of other alkanes into bilayers containing various acyl chains. Preliminary data for tetradecane–GMO bilayers give values of ΔH ≈ 1.2 kcal mol⁻¹ and ΔS ≈ 4 EU at 25 °C. The trend of the data suggests that shorter alkane molecules experience cohesive forces closer to bulk values. One reason for this is the larger volume fraction of the bilayer occupied by the smaller alkanes¹¹ which means more nearest-neighbours will be alkanes rather than acyl methyl groups. Results¹² on the solubility of hexane in various phospholipids indicates that significant differences can be expected among different types of acyl chains, and also that the interior of phospholipid bilayer dispersions is different from bulk alkyl solvents.

Finally, I should add that the results reported here for hexadecane depend on the assumptions made about how much the area per molecule of GMO varies with temperature. If the changes are large compared with those used to calculate Xₐ then ΔH and ΔS could be much larger. Using a de novo method of calculating bilayer composition which predicts relatively large changes in area per molecule¹⁶, I have calculated ΔH ≈ 9 kcal mol⁻¹ and ΔS ≈ 30 EU. Thus, the values reported here probably represent lower limits.

I thank Dr Sidney Simon for useful and enjoyable discussions and my family for their patience. The research was supported by grants from the NIH and the NSF. The generous support of the Guggenheim Multiple Sclerosis Foundation is also appreciated.

STEPHEN H. WHITE

Department of Physiology,
University of California Irvine,
Irvine, California 92717

Received April 29; accepted June 8, 1976.