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THE WATER PERMEABILITY OF THE MONOOLEIN/TRIOLEIN BILAYER MEMBRANE

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The water permeability of the lipid bilayer can be used as a probe of membrane structure. A simple model of the bilayer, the liquid hydrocarbon model, views the membrane as a thin slice of bulk hydrocarbon liquid. A previous study (Petersen, D. (1980) Biochim. Biophys. Acta 600, 666–677) showed that this model does not accurately predict the water permeability of the monoolein/*n*-hexadecane bilayer: the measured activation energy for water permeation is 50% above the predicted value. From this it was inferred that the hydrocarbon chains in the lipid bilayer are more ordered than in the bulk hydrocarbon liquid. The present study tests the liquid hydrocarbon model for the monoolein/triolein bilayer, which has been shown to contain very little triolein in the plane of the membrane (Waldbillig, R.C. and Szabo, G. (1979) Biochim. Biophys. Acta 557, 295–305). Measurements of the water permeability coefficient of the bilayer are compared with predictions of the liquid hydrocarbon model based on measurements of the water permeability coefficient of bulk 8-heptadecene. The predicted and measured values agree quite closely over the temperature range studied (15–35°C): the predicted activation energy is 11.1 ± 0.2 kcal/mol, whereas the measured activation energy for the bilayer is 9.8 ± 0.7 kcal/mol. This close agreement is in contrast with the monoolein/*n*-hexadecane results and suggests that, insofar as water permeation is concerned, the liquid hydrocarbon model quite closely represents the monoolein/triolein bilayer.

Introduction

The water permeability of the lipid bilayer can be used as a probe of membrane structure. One simple model which predicts the water permeability of the lipid bilayer is the liquid hydrocarbon model [1–3]. According to this model, the membrane is viewed simply as a thin slice of hydrocarbon liquid; water passes through the membrane by dissolving into the nonpolar interior and diffusing across it, just as it would through the bulk hydrocarbon liquid. Several studies on lipid bilayers have found water permeability coefficients in reasonable agreement with the predictions of the liquid hydrocarbon model (review, Ref. 4). However, these studies were not sufficiently precise to allow a rigorous test of the model. Recently such a test has been done [5]. This study showed that the water permeability of a monoolein/*n*-hexadecane planar bilayer membrane [6] differed significantly from the predictions of the liquid hydrocarbon model: the measured activation energy of the water permeability is 50% above the predicted value. The source of this discrepancy was inferred to be the greater degree of organization of the hydrocarbon chains in the lipid bilayer relative to the hydrocarbon liquid.

Other studies using a variety of techniques have also shown that the presence of an alkane solvent such as hexadecane results in an increase in the ordering of the acyl chains in the bilayer [7–10]. While the stability of planar lipid bilayers requires

the presence of some nonpolar solvent to satisfy the boundary conditions at the membrane annulus [11], under certain conditions the bilayer region itself is essentially solvent-free [7,12,13]. The present study was done to measure the water permeability of such a 'solvent-free' monoolein bilayer and to compare the results with the predictions of the liquid hydrocarbon model. (A preliminary acount of this work has appeared elsewhere [14].)

Methods

Generally, aliphatic hydrocarbons, typically alkanes, have been used as solvent in planar lipid bilayers [4]. Recently, however, Waldbillig and Szabo [13] showed that stable lipid bilayers could be made from pure monoacylglycerol/triacylglycerol mixtures. For this study I chose the monoolein/triolein bilayer. There are several advantages to such a choice: monoolein bilayers have been thoroughly studied with several different solvents and have yielded precise and reproducible results [7,12,15–19]; the water permeability of the monoolein/hexadecane bilayer affords a useful comparison; there is an undetectable amount of triolein solvent (less than 3% volume fraction) present in the plane of the bilayer [13]; finally, the acyl chains of triolein and monoolein are identical, so that even if some solvent is present, it should have no effect insofar as the predictions of the liquid hydrocarbon model are concerned.

Water permeability coefficient for bulk 8-heptadecene

As the alkyl chain of monoolein (or triolein) is 8-heptadecyl, 8-heptadecene is used as the liquid hydrocarbon analog of the monoolein/triolein bilayer. The water permeability coefficient of bulk 8-heptadecene is determined essentially as described previously [5]; the procedure is reviewed briefly here.

The rate of mass transport of water through a thick layer of hydrocarbon liquid is given by Fick's first law of diffusion:

$$\mathrm{d}m/\mathrm{d}t = -DA\mathrm{d}c/\mathrm{d}x \qquad (1)$$

where $\mathrm{d}m/\mathrm{d}t$ is the mass transport rate of water, D is the diffusion coefficient of water, A is the cross-sectional area of the oil layer, and $\mathrm{d}c/\mathrm{d}x$ is the concentration gradient of the water across the oil layer.

The determination of the mass transport rate of the water follows the procedure of Schatzberg [20]. A layer of oil is floated on water in a cup which is placed on the weighing pan of a recording microbalance. An identical cup filled with oil alone is placed on the taring pan. The microbalance system is maintained at essentially zero relative humidity and constant temperature. The mass transport rate of water through the oil is simply the rate of weight loss of the sample.

For the steady state achieved in the experiment the permeability coefficient of the hydrocarbon layer P_h is obtained from the mass transport rate:

$$P_h = \frac{-\mathrm{d}m/\mathrm{d}t}{Ac_w^w} \qquad (2)$$

where c_w^w is the concentration of water (mass/volume) in the aqueous phase below the hydrocarbon layer. (Note that the dimensions of P_h are distance/time.) The permeability coefficient can also be expressed in terms of the partition coefficient of water in the hydrocarbon, K, the diffusion coefficient, D, and the thickness of the hydrocarbon layer, d_h:

$$P_h = \frac{KD}{d_h} \qquad (3)$$

The liquid hydrocarbon model assumes that water permeation through the lipid bilayer occurs by exactly the same process, with the same values of K and D as in the bulk hydrocarbon liquid. Therefore, the water permeability coefficient of the bilayer membrane, P_m, can be predicted very simply from the water permeability coefficient of the bulk hydrocarbon:

$$P_m = (d_h/d_m)P_h \qquad (4)$$

where d_m is the thickness of the nonpolar interior of the bilayer membrane. The liquid hydrocarbon model ignores any contribution of the polar headgroups to the diffusion barrier for the water.

Microbalance measurements

As the microbalance measurements followed

closely the procedures given previously [5], the reader is referred to that paper for details *. The dominant experimental errors arose from uncertainties in the thickness of the 8-heptadecene layer, temperature variations during the week-long runs and evaporation losses of the 8-heptadecene. The combined uncertainty in the reported water permeability coefficients of 8-heptadecene (see Fig. 1) due to all causes is estimated to be 1–3%.

An independent check on this estimate was obtained by using results of previous measurements on the water permeability coefficient of *n*-hexadecane (Ref. 5, and unpublished data) together with the present 8-heptadecene data to 'predict' after the fact the previously measured permeability coefficients for 8-heptadecene/*n*-hexadecane mixtures [5]. Predictions and measurements agreed within combined experimental uncertainties (less than 4%) over the common temperature range (20–30°C), where it was assumed that the water permeability coefficient of the mixture is given by combining the water permeability coefficients of the pure liquids in proportion to the volume fraction of each liquid in the mixture:

$$P_{mix} = P_a\phi_a + P_b\phi_b \tag{5}$$

where ϕ_i is the volume fraction of hydrocarbon i in the mixture.

Water permeability coefficient for the monoolein/triolein bilayer

An osmotic pressure difference across a membrane leads to a flow of water through it. If the osmotic pressure difference is created by solute molecules to which the membrane is essentially impermeable (as is the case in this experiment), the volume flux of water J_v is given by [1,2]:

$$J_v = -P_v\bar{V}_w\nu(g_2c_2 - g_1c_1) \tag{6}$$

where J_v is the volume flow per unit time per unit area of membrane, $\bar{V}_w$ is the partial molar volume of water, c_i is the solute concentration in the aqueous phase on side i of the membrane, g_i is the rational osmotic coefficient associated with c_i, $\nu = 2$ when the solute is NaCl and P_f is the osmotic water permeability coefficient. If the liquid hydrocarbon model is exactly correct, P_f should agree with P_m given in Eqn. 4.

Osmotic flux measurements

The volume flow of water produced by the osmotic pressure difference across the lipid bilayer was measured in essentially the same manner as described by Hanai and Haydon [1]. Monoolein/triolein membranes were formed across a 1.5 mm diameter hole in a vertical Teflon (PTFE) septum. This septum formed the cap of a closed inner chamber machined from PCTFE. The volume of the chamber could be changed by means of a silver-plated stainless steel plunger attached to a micrometer drive (smallest division, 0.01 μl). The outer chamber was also machined from a block of PCTFE; it contained optical end windows sealed with Teflon gaskets for microscopic viewing of the membrane. An osmotic pressure difference was produced by adding a known amount of concentrated NaCl solution to the outer chamber. As water flowed out of the inner chamber in response to this osmotic shock, the membrane bowed in. This bowing was observed through a microscope (40 × magnification), and the membrane was periodically re-flattened by adjusting the micrometer. The record of micrometer readings over time gave the volume flux of water. Measurement runs typically lasted from 1 to 4 h before the membrane or experimenter broke.

Membranes were formed in a manner similar to that described by Waldbilling and Szabo [13]. Monoolein was added to 20 μl of triolein to give a final mole fraction for the monoolein of about 0.4–0.5. The mixture was warmed gently until the monoolein melted, and the liquid was then stirred with a vortex mixer. Such a mixture stayed clear for several days at room temperature. This material was applied to the hole in the Teflon septum with an acid-cleaned glass rod, the membrane thinning quickly as the rod was removed. The NaCl concentration in the outer aqueous phase was typically around 0.5 M. This solution was saturated with monoolein prior to membrane formation to prevent desorption of monoolein from the bilayer into the aqueous phase [12].

* The weighing cups were machined from blocks of poly(chlorotrifluoroethylene) (PCTFE, also known as KEL-F, Minnesota Mining and Manufacturing Co.), not poly(tetrafluoroethylene) as incorrectly stated in a previous paper [5].

The chambers were cleaned by rinsing with pure water, scrubbing with a methanol-soaked cotton swab, rinsing with petroleum ether, soaking approx. 24 h in concentrated chromic acid/sulfuric acid and a final thorough rinsing with ultrapure water.

The membrane area was determined from photographs taken through the viewing microscope which contained a two-axis graticule in the eyepiece. This graticule was calibrated against a precision optical ruling placed at the plane of the membrane. The area of the membrane was determined to a precision of better than 1% by a digital planimetric procedure. Typically, two to four photographs were taken during the course of the data collection. Membrane areas were found to be stable to about 1% during a run.

Temperature control was achieved by mounting the outer chamber and the micrometer in thermostatically controlled water jackets. A glass-enclosed thermistor in the outer chamber provided a continuous record of the temperature of the aqueous solution. The outer chamber was continuously stirred with a magnetic stirring bar. As temperature drifts can lead to a flow of water across the membrane, the membrane flatness was checked for stability before the osmotic shock was applied. Background drift rates were generally estimated to be at most 2% of the shock-induced flux. Such uncertainties are negligible compared to the observed variation in the permeability coefficeint produced by unknown causes.

Capacitance measurement

In order to predict the water permeability coefficient of the monoolein/triolein bilayer from the bulk 8-heptadecene measurements, one needs to know the thickness of the bilayer. While the thickness of the monoolein/triolein bilayer had been determined previously at room temperature [13], no data existed on the temperature-dependence of the thickness. Accordingly, membrane thickness was determined from measurements of the membrane capacitance, where the equation for the parallel-plate capacitor was used to calculate membrane thickness:

$$d_m = \epsilon_0 \epsilon / C_g \qquad (7)$$

where $\epsilon_0 = 8.854 \cdot 10^{-12}$ F/m, C_g is the membrane capacitance per unit area ('specific capacitance'), and ϵ is the dielectric coefficient of the hydrocarbon interior (see Results). Capacitance measurements were made following the procedures of White (see Ref. 12), whose apparatus was used for these measurements. The specific capacitance of the bilayer was determined with a high-precision capacitance bridge [21] driven with a 100 Hz sine wave (7 mV r.m.s.), a frequency which allows an accurate determination of the capacitance of the hydrophobic interior of the membrane [22]. A small correction was applied to remove the effect of the polarization double layers [23] present in the 0.5 M NaCl aqueous phase. Membrane area was determined in a manner similar to that described above. Temperature was maintained to ± 0.05°C during the measurements.The membrane was kept flat during the measurements by adjusting the water level in the (open) rear compartment to give a minimum capacitance. Uncertainties associated with this procedure may be the dominant source of error in the specific capacitance.

Materials

The 8-heptadecene (Chemical Samples Co.) was passed twice through an alumina column before use. As described in the previous paper [5], gas chromatography/mass spectrometry and infrared absorption measurements showed the material to be at least 99.9% pure heptadecene, with approx. 85% of the carbon-carbon double bond in the *trans* state. The monoolein (*rac*-glyceryl-1-monoolein, 99 + % pure, Supelco) was lyophilized to remove any water. A check with thin-layer chromatography showed no contamination of the monoolein. The NaCl (analytical reagant grade) was roasted at 700°C to remove organic impurities. Water was purified by distillation followed by recirculation through a system of filter, mixed-bed strong acid and base ion-exchange columns, and an activated-charcoal column (Milli-Q2 Reagant Grade Water System, Millipore Corp.). The resistivity of the output water was greater than 15 $M\Omega \cdot$ cm, and the surface tension measured with a Wilhelmy balance was 72.8 ± 0.3 dyn/cm at 20°C.

Results

The temperature dependence of the water permeability coefficient of bulk 8-heptadecene is

shown in Fig. 1. The slope of the line corresponds to an activation energy of 11.1 ± 0.2 kcal/mol *. This result is very similar to a value of 11.8 ± 0.5 kcal/mol which I had obtained previously for *n*-hexadecane [5]. A comparison of the water permeability coefficients of 8-heptadecene and *n*-hexadecane (Ref. 5; unpublished data) shows the permeability coefficients of 8-heptadecene to be 44 and 50% above the *n*-hexadecane values for 20 and 30°C, respectively. This difference probably reflects the greater solubility of water in the monounsaturated heptadecene (see Discussion).

In order to predict the permeability coefficient of the monoolein/triolein bilayer from the liquid hydrocarbon model, the thickness of the hydrophobic interior of the membrane is needed (Eqn. 4); the thickness is obtained from the specific capacitance of the membrane (Eqn. 7). The specific capacitance of the monoolein/triolein membrane for four different temperatures is given in Table I. A linear fit of capacitance vs. temperature showed the capacitance to vary at most about 1% over this temperature range. Given the experimental uncertainties, this variation was considered insignificant, and the capacitance for 20–30°C was taken as the mean value, giving 0.798 ± 0.004 (S.E.) $\mu F/cm^2$. This value is somewhat less than that given by Waldbillig and Szabo [13], 0.862 $\mu F/cm^2$, but it seems to be quite consistent with a linear interpolation of their data for the thickness of monoacylglycerol/triacylglycerol bilayer membranes vs. the number of monoacyl carbons, for 16–24 monoacyl carbons.

These results support the inference [13] that the monoolein/triolein membrane contains very little triolein solvent. The negligible change in capacitance vs. temperature indicates that the proportion of triolein in the membrane does not change over this temperature range [7], and the capacitance agrees well with White's value for the capacitance of the 'solvent-free' monoolein bilayer (obtained by freezing out the *n*-octadecane solvent), 0.790 ± 0.001 $\mu F/cm^2$ [12]. The thickness of the hydrocarbon core d_h is obtained from Eqn. 7, where the

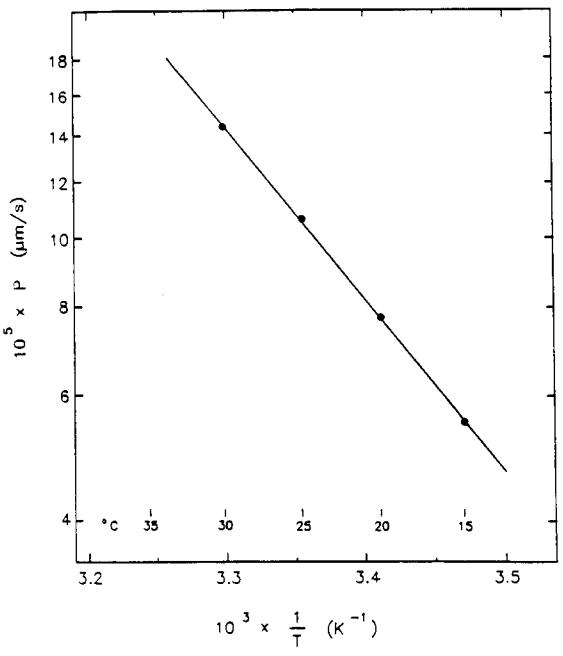

Fig. 1. Arrhenius plot of water permeability coefficient of 8-heptadecene. The permeability coefficients were obtained from the mass transport rate of water through the hydrocarbon liquid, dm/dt (see Eqn. 2), with $A = 0.500$ cm^2, $c_w^w = 1.00$ g/cm^3. Hydrocarbon thickness $d_h = 0.200$ cm. Activation energy = 11.1 ± 0.2 kcal/mol.

dielectric coefficient is taken as 2.21 [24,25]. This gives $d_h = 2.45$ nm ($\pm 2\%$), with uncertainties in both the dielectric coefficient and capacitance contributing to the uncertainty in d_h. This value for d_h agrees with the value 2.5 nm expected for a solvent-free monoolein bilayer [7,12].

Fig. 2 shows the predicted and measured water

TABLE I

SPECIFIC CAPACITANCE FOR MONOOLEIN/TRIOLEIN BILAYERS

t = temperature; C_g = specific capacitance (mean $\pm$S.D.), the number in parentheses is the number of measurements.

t (°C)	C_g ($\mu F/cm^2$)
20.3	0.789 ± 0.011 (8)
26.2	0.805 ± 0.003 (3)
29.2	0.813 ± 0.019 (4)
35.3	0.794 ± 0.006 (2)
Mean	0.798 ± 0.004 (S.E.)

* In all cases, the activation energies were obtained from a least-squares fit to an exponential function of $1/T$, $P = a \exp(b/T)$, where $b = E^{\dagger}/R$, the activation energy divided by the universal gas constant.

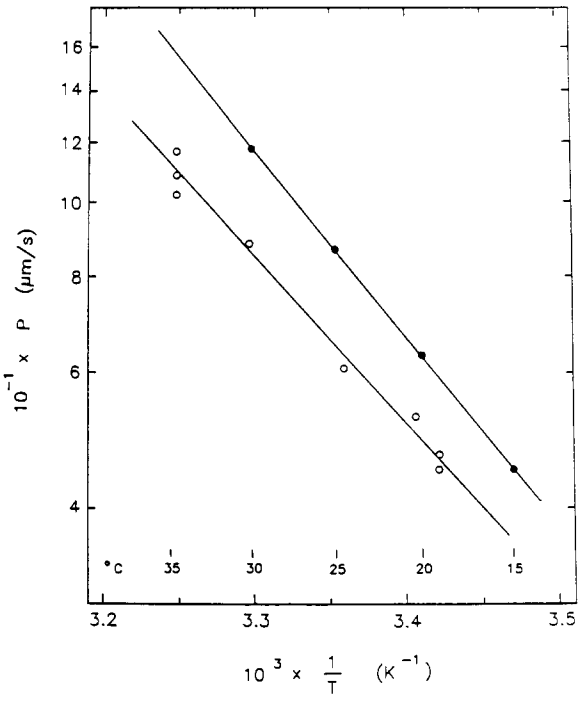

Fig. 2. Arrhenius plot of predicted and measured water permeability coefficients of the monoolein/triolein bilayer membrane. ●, predicted from liquid hydrocarbon model, Eqn. 4 and the measurements in Fig. 1, where $d_m = 2.45$ nm. Activation energy = 11.1 ± 0.2 kcal/mol. ○, measured osmotic permeability coefficients, P_f, see Eqn. 6. NaCl concentration in outer chamber, 0.35–0.77 M; NaCl concentration difference across the membrane, 0.06–0.12 M; membrane area, 0.6–1.2 mm^2. Values for rational osmotic coefficients, g_i, were obtained from the data of Harned and Owen [32]. The estimated uncertainty in P_f for any given measurement is small relative to the fluctuations from membrane to membrane shown here. Activation energy = 9.8 ± 0.7 kcal/mol.

permeability coefficients for the monoolein/triolein bilayer. The measured activation energy for water permeation of the bilayer is 9.8 ± 0.7 kcal/mol, whereas the predicted activation energy is 11.1 ± 0.2 kcal/mol. These two results are in reasonable agreement, given the uncertainties. This is to be contrasted with the significant disagreement seen between the measured and predicted activation energies obtained previously for the monoolein/*n*-hexadecane bilayer [5,6]. In order to compare these two experiments, the data for the monoolein/*n*-hexadecane bilayer should be normalized to a constant membrane thickness. When this is done, the measured and predicted activation energies for the monoolein/*n*-hexadecane bilayer are 15.6 ± 1.1 and 10.5 ± 0.3 kcal/mol, respectively. Thus, the predicted activation energy for the monoolein/*n*-hexadecane bilayer is essentially the same as the predicted and measured activation energies for the monoolein/triolein bilayer, whereas the measured activation energy for the monoolein/*n*-hexadecane bilayer is significantly larger.

Comparison of the measured and predicted water permeability coefficients for the monoolein/triolein bilayer at any given temperature shows the predicted value to be about 40% above the measured value. Unstirred layers next to the bilayer would lead to a reduction in the measured water permeability coefficient. Assuming that the liquid hydrocarbon model is precisely correct, one can calculate the thickness of the unstirred layer by noting that

$$\frac{1}{P_{meas}} = \frac{1}{P_{pred}} + \frac{d_u}{D_w} \tag{8}$$

where P_{meas} is the measured permeability coefficient, P_{pred} is the permeability coefficient predicted from the liquid hydrocarbon model, d_u is the combined thickness of the unstirred aqueous layers on both sides of the membrane, and D_w is the self-diffusion coefficient of water, $2.4 \cdot 10^{-5}$ cm^2/s at 25°C [26]. Using the 25°C data from Fig. 2 in Eqn. 8 gives $d_u = 9.6$ μm. This is to be compared with unstirred layer thicknesses of at least 70 μm for the case of the diffusion water-permeability coefficient P_d obtained from the permeation of tritiated water [3,27,28]. Correction for the unstirred layer effect for P_d resulted in $P_d = P_f$ for lipid bilayers [3,27], justifying the inference that, for osmotic permeability measurements, convective stirring brought about by density gradients near the membrane reduces the unstirred layer effect to a small correction [1,3]. Nevertheless, those experiments were not sufficiently precise to rule out the possibility of a thin (less than about 10 μm) unstirred layer in the osmotic flow measurements.

Discussion

If one assumes that there is no unstirred layer in the osmotic flow measurements, then the liquid

hydrocarbon model would have to be modified to account for the observed discrepancy between the predicted and observed water permeability coefficients for the monoolein/triolein bilayer. One approach would be to assume that the liquid hydrocarbon model works correctly for the nonpolar interior of the membrane but that the polar headgroup regions provide an additional permeation barrier (giving a correction term formally similar to the one in Eqn. 8). As indicated elsewhere [4,5], there is no unequivocal evidence in favor of such a permeation barrier, and in the following discussion the effect of polar headgroups on water permeation will be assumed to act only through their influence on the structure of the acyl chains of the lipid molecules.

Before considering the acyl chain organization of the bilayer, I want to speculate on the effect of unsaturated bonds in the hydrocarbon chains. As noted above, the water permeability coefficient of 8-heptadecene is 44–50% above that in *n*-hexadecane. (For comparison, Finkelstein and Cass [29] observed that the solubility of water in 1-heptadecene is 15% greater than in *n*-hexadecane.) The water permeability coefficient depends on both its solubility and its diffusion coefficient in the hydrocarbon liquid (see Eqn. 3). The diffusion coefficient of water in 8-heptadecene is not known. However, as the diffusion coefficient of water does not appear to be strongly dependent on the details of the hydrocarbon involved [6,20], it is reasonably safe to assume it is the same in 8-heptadecene and *n*-hexadecane. Accepting this, the above results for the water permeation through 8-heptadecene and *n*-hexadecane taken together with Schatzberg's values for the solubility of water in alkanes from C_7 to C_{16} [30] lead to the following inference: the incremental increase in water solubility (on a mole-fraction basis) due to the addition of one singly unsaturated carbon-carbon bond in the alkyl chain is about 10-times the increment produced by addition of one saturated bond. Carrying this speculation further, one can estimate the increase in water solubility in the lipid bilayer on going from monoolein (18 : 1) to monolinolein (18 : 2). This can then be compared with Fettiplace's results on the water permeability coefficients of monoolein/*n*-hexadecane and monolinolein/*n*-hexadecane bilayers [6]. In making this comparison, the contribution of *n*-hexadecane is obtained via Eqn. 5 and the measured permeabilities of the membranes are scaled to the same thickness. This estimate gives the ratio for the solubility of water in the monolinolein bilayer divided by that for monoolein bilayer as 1.29, whereas the ratio of the measured permeabilities is 1.26. This is a surprisingly good agreement, probably too good, given all the assumptions. Nevertheless, it supports the inference that the presence of unsaturated bonds in the bilayer acyl chains leads to higher water content in the membrane interior, hence to greater water permeability.

A striking result of the present experiment is the large discrepancy between the activation energy for water permeation of the monoolein/triolein bilayer, 9.8 ± 0.7 kcal/mol, and the previously measured value for the monoolein/*n*-hexadecane bilayer (scaled to a constant thickness for this comparison), 15.6 ± 1.1 kcal/mol [6]. This large difference is accompanied by an equally large discrepancy between the predicted and measured water permeation activation energies for the monoolein/*n*-hexadecane bilayer [5]. The explanation for this discrepancy appears to be that the presence of the *n*-hexadecane solvent leads to an ordering of the acyl chains in the lipid bilayer. The following summary reviews some of the arguments.

Studies of the interfacial tension of monoolein in various alkane solvents [8,16,24] have shown that the area per monoolein exposed to the aqueous phase is essentially independent of the nature of the alkane solvent. This value, 0.380 ± 0.015 nm^2, combined with the thickness of the hydrocarbon interior obtained from capacitance measurements [12,17,18] gives the total volume per lipid molecule (i.e., the volume of the lipid acyl chain plus the hydrocarbon solvent per lipid) in the bilayer. Since the acyl chain density is within a few percent of its value in bulk [31], the molecular volume of the acyl chain in the bilayer can be quite precisely determined; for the monoolein molecule this value is 0.475 nm^3 [24]. This value taken together with the total volume per lipid molecule gives the volume fraction of hydrocarbon solvent in the membrane. The fraction varies considerably with the chain length of the solvent; the nonpolar thickness of the monoolein/*n*-hexade-

cane bilayer at 25°C is 3.26–3.40 nm [7,17,18], giving a volume fraction for the hexadecane in the bilayer of about 0.25. The solvent molecules must pack into the membrane interior while maintaining the area per lipid and the membrane thickness. Thermodynamics determine this configuration. Hexadecane appears to interdigitate with the monoolein acyl chains; this leads to an increased ordering of the acyl chains normal to the bilayer surface [7,8,10]. This ordering renders the bilayer less like an isotropic hydrocarbon liquid, hence the failure of the liquid hydrocarbon model in predicting the water permeability coefficient of this bilayer membrane. [5].

In contrast, the measured activation energy for water permeation through the monoolein/triolein bilayer, 9.8 ± 0.7 kcal/mol, is in surprisingly good agreement with the value predicted from the liquid hydrocarbon model, 11.1 ± 0.2 kcal/mol. In this case, the thickness of the bilayer interior, 2.45 nm, can be combined with the molecular volume for the monoolein acyl chain (0.475 nm^3) to give an area per lipid molecule, on the assumption that no triolein solvent is present in the plane of the bilayer. The resulting value is 0.388 nm^2, in excellent agreement with the area given above for monoolein molecule in several different bilayers. This strongly supports the inference that the monoolein/triolein membrane is essentially solvent-free. The monoolein/triolein bilayer is therefore a limiting case for the monoolein bilayer: the membrane is as thin as it can be for the requisite 0.380 ± 0.015 nm^2 area per monoolein molecule. As a consequence, the lipid tails are considerably less organized than for monoolein/hexadecane. A precise determination of the molecular structure has not yet been attempted, but the water permeability results suggest that, insofar as the water permeation of the monoolein/triolein bilayer is concerned, the random configuration of the hydrocarbon chains in the bulk 8-heptadecene liquid is quite a good approximation to the configuration of the acyl chains in the monoolein/triolein bilayer.

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