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The effect of temperature on lipid–*n*-alkane interactions in lipid bilayers

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The partitioning of *n*-alkanes between an egg-phosphatidylcholine bilayer and its torus was studied for alkanes with ten to sixteen carbon atoms using measurements of membrane capacitance. The partition coefficient was found to decrease with increasing alkane chainlength and to increase with increasing temperature. This is consistent with a well-known statistical model of lipid alkane bilayers in the liquid crystalline state. It was found that *n*-decane was unsuitable as a solvent in these experiments because significant partitioning of *n*-decane into the aqueous phase and atmosphere occurred and this could not be adequately controlled. Egg-phosphatidylcholine bilayers containing negligible amounts of solvent could be produced using a method similar to the 'freeze-out' method of White (Biochim. Biophys. Acta 356 (1974) 8–16). Bilayers formed using *n*-hexadecane were found to be virtually solvent-free at temperatures below 30°C. The partition coefficient of *n*-alkanes in the bilayer was also found to depend on the alkane mole fraction. Thus it was concluded that the assumption of ideal mixing between acyl and alkane chains in the bilayer was not valid when the alkane mole fraction exceeded 40% (with respect to the acyl chains of the lipid). The variation of the standard chemical potential with temperature was measured for alkanes of different chainlengths and it was concluded that the enthalpy and entropy of the alkanes in the bilayer are in themselves temperature-dependent. This indicates that the state of the hydrophobic interior of lipid bilayers varies with temperature.

Introduction

In order to model the physical and chemical properties of living membranes, artificial lipid bilayer systems such as vesicles, planar bilayers and multilayers have been extensively studied. Single, planar lipid bilayers, in principle, should accurately model the bilayer component of living membranes. A common method of forming planar bilayers is to disperse the lipids in a hydrophobic solvent such as one of the *n*-alkanes. A film of this solution is then established across an aperture in a septum dividing two aqueous solutions. The bilayer spontaneously forms as the solvent is expelled from the film into the surrounding annular region (torus) between the bilayer and the septum (see for example, Ref. 1). The bilayers so formed [§] contain varying amounts of alkane solvent which in the steady-state is presumably in thermodynamic equilibrium with the bulk lipid-alkane solution of the torus and the lipid in the aqueous solution.

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§ It should be noted here that bilayers formed by the alternative technique of monolayer apposition also require small amounts of solvent present during formation to maintain stability [1,4,5].

Naturally occurring membranes probably do not contain any alkane hydrophobic molecules. Further, the incorporation of solvents such as the alkanes into living membranes significantly alters membrane function; they act indiscriminately as local anaesthetics [2].

The presence of varying concentrations of *n*-alkanes has also been reported to modulate the conduction properties of reconstituted membranes containing passive ion channels such as those formed by the addition of gramicidin into the membrane [3].

In the past, differences in the response of multilayer lipid preparations and single planar bilayers to the addition of molecules such as benzyl alcohol and cholesterol have been attributed in part to the presence of *n*-alkane solvents in the latter (e.g. Ref. 6). It was also suggested that these molecules may induce changes in the partitioning of *n*-alkanes into the bilayer interior [2,6]. Knowledge concerning the interaction between hydrophobic solvents, such as the *n*-alkanes, and lipid bilayers is thus also important to our understanding of this membrane model.

In order to identify the factors which modulate the solubility of alkanes in lipid bilayers, the temperature-dependent partitioning of *n*-alkanes (chainlength 10 to 16 carbon atoms) between the bilayer and torus was investigated in this study.

The absorption of alkanes into the interior of artificial planar bilayers is known to increase their thickness [5,7,8]. By assuming that the alkane concentration in the bilayer was linearly related to the membrane thickness, the alkane concentration in the bilayer could be determined (Refs. 7,8 see also Appendix). Studies of alkane absorption in bilayers formed from monoacylglycerols such as glycerol monooleate have shown that the concentration of alkane solvent present depends on the chainlength of both the monoacylglycerol and the alkane components of the bilayer [7,8]. White [9] found that bilayers could be formed with negligibly small (equilibrium) solvent concentrations by the use of hydrophobic solvents that have such large molecular dimensions (e.g., squalene), that they are effectively too big to fit into the hydrophobic part of the bilayer structure (i.e., they are similar in length to the hydrocarbon chains of the monoacylglycerol). Independent Raman spectroscopy studies [10] support White's conclusion. Further, it has been found that the relative solubility of alkane in the bilayer is also dependent on temperature [8]. Decreasing the temperature causes the solvent in the bilayer to collect into (perhaps frozen) microlenses, whereupon it is effectively removed from the bilayer proper. This effect has been exploited by White [11] as a method for producing essentially solventless glycerol monooleate bilayers. In this paper we report an extension of such alkane solubility experiments to egg-phosphatidylcholine bilayers.

A statistical mechanical (mean field) model of the acyl chain conformations was developed by Marčelja [12]. Gruen [13] refined the mean field model of Marčelja by including explicit terms in the partition function which accounted for the polar group interactions and the oil/water surface free energy at the bilayer/water interface. An extension of this model [14,15] gave the partitioning of alkanes between a saturated aqueous phase and the bilayer interior. The results presented here are in qualitative agreement with those predicted by Gruen's model of the bilayer interior.

Materials and Methods

Bilayers were generated from solutions of egg-phosphatidylcholine dissolved in *n*-alkanes (15 mM with respect to phosphatidylcholine) with chainlengths between ten carbon atoms (*n*-decane) and sixteen carbon atoms (*n*-hexadecane). Bilayers were generated at temperatures between 25 and 45°C (depending on the alkane solvent) with 1 mM KCl in the aqueous phase.

The mole fraction of *n*-alkanes within the hydrophobic region of each lipid bilayer was calculated from four-terminal measurements of its total capacitance at a frequency of 1 Hz (see Appendix). The details of this technique and apparatus have been discussed elsewhere [16–19].

Formation of bilayers from two-component solutions (i.e., phosphatidylcholine and alkane) of some of the alkanes at very low concentrations of the alkane could not be achieved because of the limited solubility of lipids in that alkane. This problem was overcome by dissolving the lipids in a two-component solvent; i.e., the alkane under

study (e.g., dodecane) plus hexadecane (i.e., a three-component solution). The hexadecane in which the lipid was dissolved, at temperatures less than 35°C, was essentially excluded from the bilayer phase. The effect of low concentrations of shorter-chainlength alkanes could then be studied by adding small amounts of the shorter-chainlength alkanes to the lipid/hexadecane mixture.

In these studies, the bilayer and torus were assumed to be close to equilibrium when the capacitance of the membrane had attained a steady value (varying less than 1% in 15 min). All measurements reported here are for bilayers which had been allowed to come to such thermodynamic equilibrium with the torus and were 'black' over virtually the entire aperture in the septum. Care was taken to ensure that the bilayer remained planar during capacitance measurements, by periodically adjusting the hydrostatic pressure across the film. The absolute value of the bilayer area, excluding that of the torus, was determined (to an accuracy of 2%) using a eyepiece graticule mounted in a 40 × microscope.

Results

Stable egg-phosphatidylcholine bilayers could be generated only at temperatures which were sufficiently high to ensure that initially significant amounts of alkane solvent remained in the bilayer immediately after formation of the 'black' membrane (at least 1 : 10 mole ratio alkane : egg-phosphatidylcholine). Only after the film had become bimolecular over the entire aperture in the septum could the temperature be lowered. Upon lowering the temperature, solvent left the bilayer phase of the membrane, apparently aggregating into microlenses (i.e., small droplets of liquid alkane visible as isolated 'pinpoints' of high reflectance under the viewing microscope [20]). The membrane would usually attain a steady capacitance within 15–20 min of a change in temperature.

Bilayer capacitance; temperature-dependence

It can be seen from Fig. 1 that the upper limiting value of the capacitance at low temperatures of all bilayers generated from solutions of the lipid for the longer chainlength alkanes (C_{14}–C_{16} alkanes) was similar at low temperatures. The bilayer capacitance decreased with increasing temperature, asymptotically approaching a lower limit of approx. 3.5 ± 0.3 mF/m^2. From the results shown in Fig. 1 it is clear that, at any given temperature, the bilayer thickness (and hence alkane solubility) was reduced as the chainlength of the alkane present was increased.

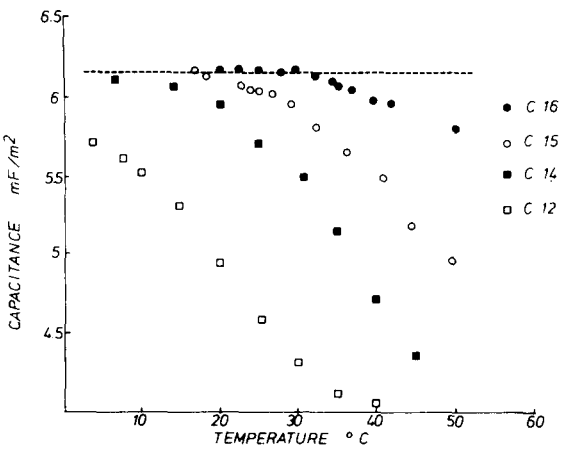

Fig. 1. The capacitance, measured at 1 Hz, of representative bilayers in equilibrium with egg-phosphatidylcholine solutions containing different chainlength alkanes at different temperatures. The horizontal dashed line is the low temperature upper limit to membrane capacitance, which is common to all the egg-phosphatidylcholine bilayers formed from C_{14}–C_{16} alkane solution in 1 mM KCl over the temperature range employed here.

Bilayer capacitance: dependence on torus alkane concentration

Egg-phosphatidylcholine bilayers were formed from solutions in which the concentration of a given alkane molecule could be varied over a wide range (see Materials and Methods). The capacitance of egg-phosphatidylcholine bilayers formed with a mixture of *n*-decane and *n*-hexadecane was found to have no well-defined, stable value, and typically varied between 3.7 and 5 mF/m^2 over a period of 2 h. This long-term variation in capacitance was not present in bilayers formed from longer-chainlength alkanes such as *n*-dodecane.

Inspection of Fig. 2 shows that the total scatter in the bilayer capacitance between different bilayers (for a given solvent) increased with increasing alkane concentration in the bilayer. The scatter in the bilayer capacitance varied from 4%, when

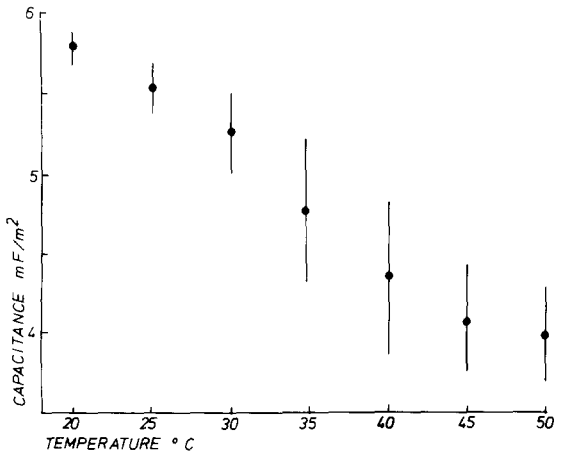

Fig. 2. The capacitance of lipid bilayers in equilibrium with *n*-tetradecane solutions of egg-phosphatidylcholine in 1 mM KCl. The error bars refer to the variation in the measured capacitance of ten egg-phosphatidylcholine bilayers. Note that the scatter increases with decreasing membrane capacitance.

essentially no solvent was present, to a maximum of 20%.

The temperature-dependent capacitance of bilayers in equilibrium with different mole fractions of *n*-dodecane in the torus is shown in Fig. 3. It was found that a decrease in the mole fraction of *n*-dodecane in the torus caused an increase in the temperature-dependent, bilayer capacitance.

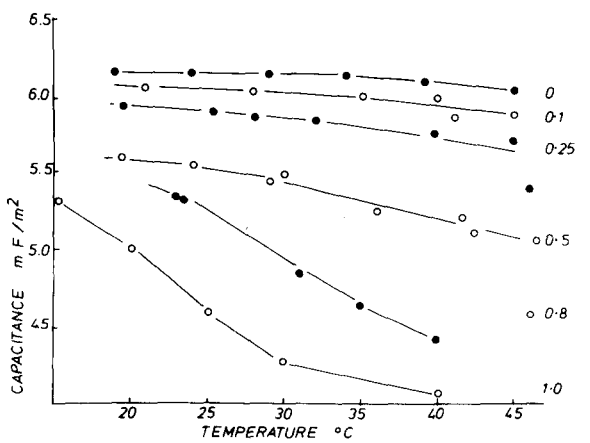

Fig. 3. The capacitance of egg-phosphatidylcholine bilayers in equilibrium with solutions containing various mixtures of *n*-hexadecane and *n*-dodecane in 1 mM KCl. The numbers at the right-hand side of the graph indicate the mole fraction of *n*-dodecane in the membrane-forming solution.

Discussion

Interpretation of the low-temperature capacitance

The capacitance measurements showed that alkanes of different chainlengths had different, temperature-dependent, effects on the thickness of planar lipid bilayer membranes. These observations, including the dependence on chainlength, are consistent with some X-ray diffraction measurements (e.g., Ref. 41)). However, other X-ray and neutron diffraction studies (e.g., White et al. [27]) have shown that the thickness of membranes in multilayer preparations used in such diffraction studies is not dependent on the amount of alkane absorbed into the preparations; the absorption of alkane molecules in these systems was apparently accompanied by an appropriate change in the area per lipid molecule. This curious dichotomy in the effects of alkane on planar bilayers and in some multilayer preparations has been noted and discussed previously. Thus, White et al. [27] have suggested that the disparate effects are related to the depressed value of the water activity in the multi-layer preparations relative to those in planar bilayer preparations where the water activity coefficient is essentially unity.

The capacitance measurements revealed that the thickness of the planar bilayer membranes was modulated by the absorption of alkane from the torus. However, at sufficiently low temperatures, the differential effects of alkanes with different chainlengths vanished and the capacitances (and hence the thickness) of the bilayers were independent of alkane chainlength and temperature (at least for the longer-chainlength alkanes). This suggests that the concentration of the longer-chainlength alkanes in the membrane at low temperatures must be very small. The data presented suggest that this pattern for the effect of temperature is also true for *n*-dodecane, except that the process was incomplete at the lowest temperature that could be used (see Fig. 1).

The capacitance of egg-phosphatidylcholine bilayers formed by this method in 100 mM KCl was 6.8 ± 0.2 mF/m^2 at 25°C, which compared favourably with the capacitance of bilayers formed by monolayer apposition (7.21 ± 0.2 mF/m^2). The latter are believed to have a negligible solvent concentration as determined by the effect of d.c.

voltage bias on the membrane capacitance [5].

Based on these data, it is now assumed that bilayers containing *n*-hexadecane in 1 mM KCl external electrolyte are essentially solvent-free * at temperatures up to 30°C and that the upper limiting capacitance at low temperatures is the capacitance of the solvent-free bilayer.

The partition coefficient, K, of *n*-alkane chains between the bilayer and torus of lipid membranes is numerically equal to the mole fraction of alkanes in the bilayer provided that the alkane mole fraction in the torus is unity §.

Solventless egg-phosphatidylcholine bilayers could be produced by generating the bilayer films at elevated temperatures and then reducing the temperature which lowered the alkane concentration. This technique is analogous to the 'freeze-out' method of White [11].

Effect of microlenses

White [11] observed that alkane which was displaced from the bilayer upon changes in the bilayer-torus equilibrium collected into small lenses of bulk alkane which he referred to as microlenses and which were clearly visible under a low-power microscope.

This phenomenon was also observed in the present experiments. Being much thicker than the surrounding bilayer, the lenses of trapped solvent contribute little to the capacitance of the bilayer. If the microlenses occupied a significant fraction of the membrane surface area, this would introduce errors into the estimates of bilayer thickness derived from the total membrane capacitance. This, however, is not the case.

A detailed study of the effect of microlenses on estimates of the bilayer area has been carried out by White and Thompson [20]. It was found that the total area occupied by microlenses depended on the amount of solvent disproportioned from the bilayer and the size of the microlenses formed. The calculation of White and Thompson showed that in extreme cases microlenses could occupy 10% of the bilayer area. The presence of microlenses in lipid bilayers was believed to be the cause of the considerable scatter in the bilayer capacitance observed in their experiments.

In the present study for bilayers containing appreciable amounts of alkane, the scatter in the capacitance of bilayers was similar to that reported by White and Thompson for bilayers containing *n*-decane. However, if variations in the area of microlenses between different membranes were responsible for the experimentally observed scatter, then large variations in the limiting (upper) value of the capacitance would also have been observed. This was not the case. Therefore it can be concluded that microlenses only had a small effect on the total capacitance of the membrane (at most 2%). The experimental scatter must then arise from variations of up to 25% in the alkane concentrations within those bilayers which contain significant alkane in the interior of the membrane. The reason for the considerable variability is not understood.

Bilayers formed from binary mixtures of n-alkanes

Nearly all of the earlier published bilayer work has been on egg-phosphatidylcholine bilayers formed from *n*-decane solutions. As found in many previous studies, bilayers formed from *n*-decane solutions had no well defined capacitance (e.g. see Refs. 20–23). In some of these studies [20,21] it was suggested that this was due to a time-varying disproportioning of *n*-decane into microlenses. In the present experiments it was apparent that *n*-decane was sufficiently volatile to affect the time-course of the capacitance of bilayers formed from this solvent, an effect already known in bilayers containing *n*-nonane [2]. Thus, the bilayer thickness never reached a stable value in the life-time of the membrane (typically 2 h). Therefore, the bilayers containing *n*-decane must be treated as three-phase systems (i.e., the bilayer, the torus and the atmosphere) and precautions would need to be taken to ensure that the aqueous phase and adjacent atmosphere are in equilibrium with the *n*-decane in the bilayer (for the atmosphere this represents substantial technical problems). The fact that such precautions were in general not taken may account for the considerable variation in

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* Solvent-free only in the sense that the bilayer thickness was unaffected (within the experimental error of ±22%) by the possible presence of trace amounts of solvent within the bilayer.

§ The lipid concentration in the torus was always very small (under 30 mM). The total alkane mole fraction was then always greater than 0.99.

bilayer capacitances reported in the literature for apparently identical bilayer systems using *n*-decane solvents (e.g., the capacitance of egg-phosphatidylcholine–cholesterol bilayers containing *n*-decane; cf. Refs. 2, 24).

The assumption of ideal mixing between the n-alkane and acyl chains

Provided ideal mixing occurs between the alkane and lipid acyl chains, the standard chemical potential difference, $\Delta\mu^\circ$, and the partition coefficient of *n*-alkanes, K, between the bilayer and torus phases are related by Eqn. 1:

$$\Delta\mu^\circ = -RT \ln K \quad (1)$$

where T is the absolute temperature and R is the molar gas constant. The standard chemical potential difference can be expressed in terms of a difference in the internal entropy, ΔS°, and enthalpy, ΔH°, of the *n*-alkane. Thus:

$$\Delta\mu^\circ = \Delta H^\circ - T\Delta S^\circ \quad (2)$$

Considerable evidence exists to show that the alkane is not evenly distributed throughout the bilayer interior, and that the alkane preferentially occupies the region near the midplane of the bilayer; the region near the bilayer/water interface being effectively inaccessible to alkanes (e.g. Refs. 25–27). It has been calculated [15] that at low alkane concentrations the alkane molecules are distributed fairly uniformly throughout the hydrophobic interior, since their mixing entropy overshadows the decrease in configurational entropy imposed by the order of the acyl chains (see next section). At higher alkane concentrations the *n*-alkane distribution is non-uniform. The alkane mole fraction approaches unity near the bilayer midplane, whereas that near the bilayer/water interface is low [15]; i.e., the mixing of acyl and alkane chains deviates significantly from ideality. If this is correct, then the thermodynamic analysis presented here as well as in some previous studies (see for example, Refs. 2 and 8) would not necessarily be applicable to this system.

To check this, the partition coefficient of *n*-dodecane was measured as a function of its mole fraction in the bilayer by varying the alkane mole fraction in the torus at constant temperature. Results for this are shown in Fig. 4. The partition coefficient of *n*-dodecane between the bilayer and torus was independent of *n*-dodecane mole fractions in the bilayer up to 0.4. After this, the partition coefficient decreased with increasing concentrations in the bilayer. Thus, for alkane mole fractions in the bilayer less than 0.4, the assumption of ideal mixing of acyl chains and alkanes is not a bad approximation. The decrease observed is due to the fact that further partitioning then largely occurs into the alkane chains near the bilayer surface, where $\Delta\mu^\circ$ is higher.

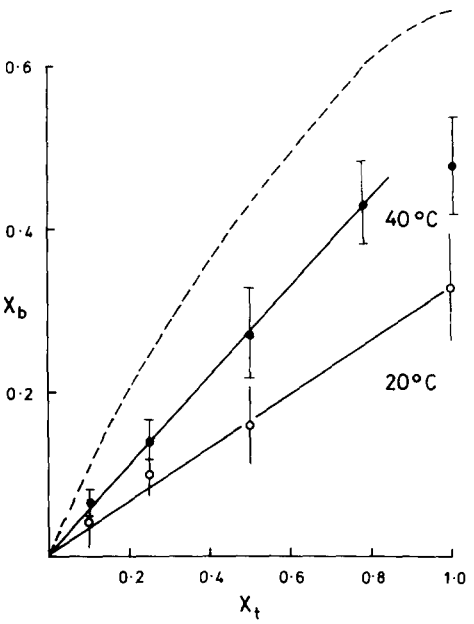

Fig. 4. The mole fraction of *n*-dodecane in egg-phosphatidylcholine bilayers (X_b) plotted against the mole fraction of *n*-dodecane in the membrane forming solution (X_t) at 20°C and 40°C. The data presented in this figure were calculated from the capacitance data in Fig. 3. The dashed line is the mole fraction of *n*-dodecane near the bilayer midplane, calculated from the data at 40°C, assuming that the dodecane was only distributed throughout 50% of the bilayer interior. Note the relation between X_b and X_t is essentially linear up to values of X_t of approx. 0.4.

The bilayer interior; order and its effect on n-alkane partitioning

The acyl chains in a lipid bilayer above its phase transition are in a semi-ordered state. Order is imparted to them by their attachment at one end to polar head-groups which sit at the bilayer/water interface. The acyl chains are par-

tially straightened to reduce high-energy hydrocarbon-water contact.

The alkanes are chemically similar to the acyl chains. However, the alkanes have no polar groups and therefore are not anchored to the bilayer interface and are free to reside wholly within the bilayer. Gruen [14], when modelling the absorption of alkanes in lipid bilayers, considered the free-energy cost of creating space for the alkanes and the free energy of mixing of acyl and alkane chains in the plane of the bilayer.

Gruen's [13] modelling of this system predicted two important factors which affect the alkane absorption and which are both related to the order parameter * of the acyl chains [14,15].

Firstly, in regions of high acyl-chain order the alkane chains are partially constrained to lie parallel to the acyl chains. Thus, the internal entropy of the alkane molecules is much lower in these regions than in regions of low acyl-chain order. Hence, regions of high acyl-chain order in the bilayer are relatively hostile to the presence of alkanes. Additionally, if alkane molecules were intercalated into the lipid near the hydrophobic/polar head interface, the acyl chains would need to become further ordered in order to minimize the increase in interfacial free energy which must result from the increased lipid-lipid spacing which follows from inserting an alkane molecule in this region.

A consequence of this is that, while shorter-chainlength solvents would be able to partition into the hydrophobic region near the bilayer midplane, longer-chainlength alkanes, being partly constrained to lie parallel to the acyl chains, would have a portion of their structure located in the more ordered outer part of the hydrophobic region of the bilayer and so would not be able to partition into the bilayer as easily.

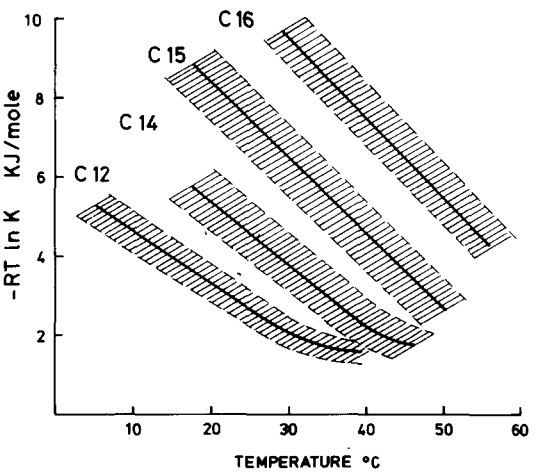

Fig. 5. The difference in the standard chemical potential between the bilayer and torus for different chainlength alkanes at different temperatures. The heavy central lines indicate the typical temperature-dependence of $\Delta\mu^\circ$ for a given bilayer. $\Delta\mu^\circ$ was calculated from data shown in Fig. 1 using Eqns. 1, A2 and A3. The shaded area represents the scatter over 5–10 different membranes. The main variation in the temperature-dependence of $\Delta\mu^\circ$ between different membranes was due to variation in the intercept rather than the slope of the temperature-dependence.

In spite of the significant experimental scatter, the chainlength-dependence of alkane absorption in egg-phosphatidylcholine bilayers was found to be quite significant and was consistent with previous measurements on egg-phosphatidylcholine bilayers [7] and similar to that found in glycerol monooleate bilayers [5,8]. From Fig. 5 it can be seen that the chainlength dependence of $\Delta\mu^\circ$ is very pronounced, increasing by 2.5 kJ/mol for each additional carbon atom in the alkane chain.

The low values of $\Delta\mu^\circ$ for *n*-dodecane show that alkane near the midplane of the bilayer contributes very little to the $\Delta\mu^\circ$ of the longer alkanes. This suggests that the main contribution to the standard chemical potential of the alkanes in the bilayer arises from the terminal carbon atoms of the longer alkanes. Thus it seems that the partitioning of *n*-alkanes into the bilayer would be sensitive to the order parameters of the acyl chains near the bilayer/water interface.

* The order parameter, n_i, at the ith carbon atom of the acyl chain is defined by the expression:

$$n_i = \langle \tfrac{3}{2}\cos^2\theta_i - \tfrac{1}{2} \rangle$$

Where 'θ' is the angle between the local acyl-chain axis and the bilayer normal. The parentheses indicate a thermodynamic average. The order parameter is a measure of the orientational order of the chains. A larger order parameter implies a high degree of alignment and hence a small array of conformations available to the chains. Thus, large order parameters effectively imply a small internal entropy of the acyl chains.

Absorption of n-alkanes: interpretation of temperature-dependence

The temperature-dependence of $\Delta\mu°$, derived from Eqn. 2, is given by:

$$\frac{\partial\Delta\mu°}{\partial T} = \frac{\partial\Delta H°}{\partial T} - \Delta S° - T\frac{\partial\Delta S°}{\partial T} \quad (3)$$

In some previous studies [7,8], it has been assumed that $\Delta S°$ and $\Delta H°$ are temperature-independent. Hence, Eqns. 2 and 3 reduce to the following well-known expressions:

$$\Delta S° = -\frac{\partial\Delta\mu°}{\partial T} \quad (4)$$

$$\Delta H° = [\Delta\mu°]_{T=0} \quad (5)$$

Fig. 6 shows the temperature-dependence of the difference in the standard chemical potential, $\Delta\mu°$, in the bilayer and torus for different alkanes. The temperature-dependence increased with increasing chainlength of the alkane solvent. At higher temperatures, the temperature-dependence of $\Delta\mu°$ appeared to vanish (at least for the shorter-chain-length alkanes). ΔS, obtained from Fig. 6 using Eqn. 4 for the different alkanes studied, was about 200 $J \cdot mol^{-1} \cdot K^{-1}$, which is certainly a considerable overestimate of the likely value, as the state of the bilayer interior is not very different from that in the membrane torus. The value for $\Delta S°$ presented here is nearly an order of magnitude greater than the entropy of fusion of alkanes.

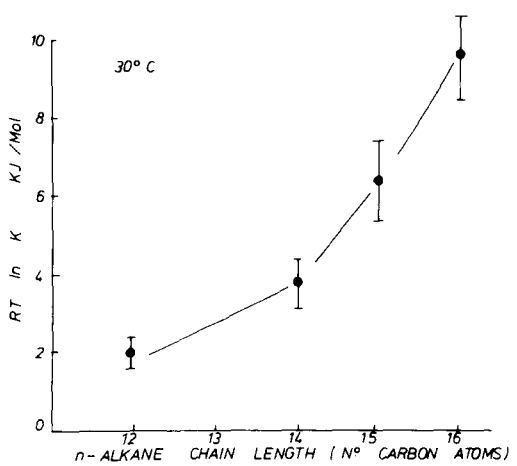

Fig. 6. The difference in the standard chemical potential between alkanes in the bilayer and torus phases of the membrane for alkanes of different chainlengths, at 30°C. $\Delta\mu°$ was calculated from the data shown in Fig. 1 using Eqns. A3 and 4.

According to Gruen [15], $\Delta H°$ and $\Delta S°$ for alkane partitioning are dependent on the ordering of the lipid acyl chains in the bilayer. Deuterated lipid NMR studies [30] on egg-phosphatidylcholine multilayer preparations show that the order parameter of perdeuterostearic acid intercalated in the acyl chains is temperature-dependent, decreasing from 0.4 to 0.36 between 30°C and 50°C. Thus, it seems that most of the temperature dependence of $\Delta\mu°$ is a result of the temperature-dependent acyl chain order in the bilayer interior, that Eqns. 4 and 5 are not applicable to alkane lipid bilayer systems studied here, and that setting $\partial\Delta H°/\partial T$ and $\partial\Delta S°/\partial T$ to zero in Eqn. 3 is not a good approximation.

Appendix

Calculation of n-alkane concentration in egg-phosphatidylcholine bilayers

It has been shown that bilayer membranes containing alkane solvents are thicker than those that are solvent-free. Provided the difference in thickness, Δd, between the solvent-free and solvent-containing bilayer arises entirely from the partial molar volume of the alkane in the bilayer (i.e., provided the area density of the lipids is unchanged), one can calculate the molar concentration of alkane per unit area in the bilayer, c_a, using the following expression:

$$c_a = \frac{\Delta d\rho}{M} \quad (A1)$$

where ρ and M are the mass density and molecular weight of the solvent. The volume-averaged mole fraction of alkane with respect to the acyl chains, X_a, is given by:

$$X_a = \frac{c_a}{c_a + c_l} \quad (A2)$$

where c_l is the number of moles of acyl chains per unit of membrane area.

Previous studies (e.g. Refs. 4, 7, 11) have used measurements of membrane capacitance to measure Δd and hence estimate the molar concentra-

tion per unit area in the bilayer. In this study, Δd was calculated using the following expression:

$$\Delta d = \varepsilon_0 \varepsilon_r \left[\frac{1}{C} - \frac{1}{C'} \right] \quad \text{(A3)}$$

where C and C′ are the capacitances of the alkane-lipid bilayer and the same bilayer in its alkane free state, respectively. ε_0 and ε_r are the permittivity of free space and relative dielectric constant of the hydrophobic region, which is about 2.1–2.13 [31]. Eqn. A3 is valid provided that:

(a) The molecular volume of the alkane solvent contributes to the volume of the hydrophobic region without contributing to the membrane surface area, as an increase in the membrane area per molecule is energetically unfavourable. Thus, the bilayer interfacial area occupied by each lipid is considered independent of both temperature * and alkane concentration (see Ref. 4).

(b) The dielectric constant of the alkanes used in these experiments was in the range 2.02–2.06, which was considered to be approximately equal (within error of ±2%) to that of the lipid acyl chains. The temperature-dependence of the dielectric constant is negligible; varying less than ±2% in the temperature range of the experiments [32].

(c) The alkane volume density is temperature-independent in the temperature range of these experiments, to within 3% (e.g., Ref. 33).

(d) The error introduced by neglecting the effect of microlenses on the measured area of these bilayers is small (Ref. 20, see also Discussion).

The thickness of the solvent-free bilayer is largely determined by the ratio of the partial molar volume of the acyl chains of the molecules to the area of the lipid molecule in the plane of the bilayer [34]. The former quantity can be considered constant.

Previous measurements [23,35] show that the capacitance of egg-phosphatidylcholine bilayers at frequencies of 1 Hz includes the geometric capacitance of the central hydrophobic layer, as well as the series capacitance of the carboxyl-ester-oxygens on either side of the membrane. Further, it is known that the capacitance of the Gouy-Chapman ionic double layers on either side of the membrane also contributes to the measured capacitance at 1 Hz, although this should be significant only at low ion concentrations in the aqueous phase [23,36]. Discussion of the effects of ionic double layers on the measured bilayer capacitance is not presented here. For further details, the reader is referred to the discussion in Refs. 36–39. However, on inspection of Eqn. A3, one can see that any errors introduced by ignoring the effects of bilayer substructure or ion double layers cancel exactly when calculating the alkane concentration within the bilayer.

* Increasing the temperature, if anything, should on entropic grounds, cause a (small) increase in area occupied per lipid molecule.

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