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A THERMODYNAMIC STUDY OF THE PARTITION OF *n*-HEXANE INTO PHOSPHATIDYLCHOLINE AND PHOSPHATIDYLCHOLINE-CHOLESTEROL BILAYERS

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Summary

The partition coefficient of *n*-hexane from water to bilayers of dioleoyl phosphatidylcholine and egg lecithin were measured as a function of temperature. Over a 50°C temperature range, the partition coefficient decreased linearly with temperature. The addition of cholesterol (1 : 1 mol ratio) to those bilayers reduced the partition coefficient by, at most, a factor of 2.5 at 25°C.

The thermodynamic transfer parameters so obtained were compared to those of bulk hydrocarbon liquids and sodium dodecyl sulphate micelles. The results indicate that a bulk hydrocarbon liquid is not a good model for the interior of a bilayer whereas sodium dodecyl sulphate has approximately the same thermodynamic transfer parameters as egg lecithin.

A comparison of the thermodynamic transfer parameters of *n*-hexane from a hydrocarbon liquid to either egg lecithin or dioleoyl phosphatidylcholine at 25°C shows that energetically and entropically the *n*-hexane prefers the bulk hydrocarbon phase whereas enthalpically it prefers the bilayer. These data imply that the *n*-hexane is aligned, on the average, parallel to the acyl chains.

The partition coefficient, K, of *n*-hexane from water into various solvents follows the sequence $K_{HC} > K_{EL} \approx K_{SDS} \geqslant K_{DP} > K_{DPC} > K_{Oct}$, where the subscript HC represents hydrocarbon; EL, egg lecithin; SDS, sodium dodecyl sulfate; DP, dioleoyl phosphatidylcholine; DPC, dioleoyl phosphatidylcholine cholesterol; and Oct, *n*-octanol.

Introduction

The interactions of well-characterized molecules with biological membranes is of current interest in membrane biophysics. Historically it has been found

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Abbreviations: EL, egg lecithin; DP, dioleoyl phosphatidylcholine; DPC, dioleoyl phosphatidylcholine-cholesterol; SDS, sodium dodecyl sulphate; BL, bilayer; HC, hydrocarbon; Oct, *n*-octanol; C, cholesterol.

that the permeability of a given class of molecules through membranes is related to their solubility into an organic liquid such as olive oil [1]. As the membrane permeability is directly proportional to the membrane/water partition coefficient, one could measure the partition coefficient in varous organic liquids and permeability through membranes to ascertain which organic solvent "most closely" resembled the biological membrane of interest. Similar studies could be done correlating anesthetic potency with solubility in various organic liquids [2]. Investigations of this type revealed that bulk organic liquids such as olive oil, ethyl ether and *n*-octanol can be used as model solvents [2—7]. Many of those solvents exhibit similar selectivity sequences to a given series of molecules as did the biological system being investigated [2].

The objective of this paper is to ascertain how valid a model is a bulk hydrocarbon liquid for a lipid bilayer and consequently a biological membrane. To determine any differences we have directly measured the membrane/water partition coefficient of *n*-hexane into lipid bilayers of different compositions and compared those results to the partition coefficients of *n*-hexane into organic liquids.

We found that the free energy of transfer of *n*-hexane from a bulk hydrocarbon solvent to a lipid bilayer is not energetically favored. Enthalpically *n*-hexane prefers the lipid bilayer whereas entropically it prefers the bulk hydrocarbon phase. The addition of cholesterol reduces the partition coefficient by a factor of about 2.5.

We conclude that for a molecule like *n*-hexane a bulk hydrocarbon liquid is not a good model for a lipid bilayer.

Materials and Methods

Dioleoyl phosphatidylcholine and egg lecithin were obtained from Supelco Inc. and used as obtained. They gave a single spot on thin-layer chromatography. Cholesterol was obtained from Applied Science and used as obtained. Lipid dispersions were obtained by vortexing the phosphatidylcholine in a nitrogen-saturated 0.1 M NaCl solution (to give a concentration of about 10 mg/ml) for about 1 min. The concentration was determined by a Bartlett phosphorous assay [10,11]. The water was twice distilled and the salt was washed with chloroform to remove non-polar impurities. The phosphatidylcholin-cholesterol vesicles were prepared by dissolving both components in chloroform, removing the solvent and then proceeding as above. *n*-[1-^{14}C]-Hexane was purchased from ICN Isotope and Nuclear Division (lot number 557158). This radioactive hexane was diluted with pure *n*-hexane and its specific activity determined.

The partition coefficient of *n*-hexane between water and bilayers was measured by a gas solubility cell as described by Wishnia and Pinder [8]. The procedure is explained in detail elsewhere [9]. The cell is an all glass apparatus having four interconnecting chambers that permit exchange of gas but not the liquid phases. The first and fourth chambers contained water whereas the middle two contained lipid dispersions. This closed system is enclosed in a bottle through which water at regulated temperatures can flow. The temperature was measured by a thermistor of the water leaving the cell which is

accurate to ±0.3° C. Experiments were performed both on heating and cooling cycles. Similar results were obtained.

The radioactive hexane was introduced into the cell in the liquid phase via a microliter syringe that punctured a serum stopper. The samples were obtained by taking 80-μl samples from each chamber. The number of counts in each chamber remained constant after about 30 min of mixing. Counting was done on a Beckman LS-100 counter. The counts per min in the aqueous and lipid phases are straightforwardly related to the mol fraction in each phase. The units used in this paper are unitary free energy units [12].

Results

Dioleoyl phosphatidylcholine and egg lecithin bilayers

The partition coefficient of *n*-hexane into dispersions of dioleoyl phosphatidylcholine at 25° C is independent of the aqueous concentrations of *n*-hexane in the range $1.1 \cdot 10^{-7} < X_w < 19.7 \cdot 10^{-7}$ as described by Eqn. 1.

$$X_L(10^3) = 0.964 + 3.175\, X_w(10^7);\ r^2 = 0.97 \qquad (1)$$

The mol fraction of *n*-hexane X_L, in the dioleoyl phosphatidylcholine dispersions insures that we have a dilute solution of *n*-hexane in dioleoyl phosphatidylcholine. In these dilute solutions, the entropy of mixing is going to be nearly ideal [13] although the heat of mixing of *n*-hexane with dioleoyl phosphatidylcholine (or egg lecithin) may be non-ideal. The independence of the partition coefficient on aqueous concentration was also found by Stone [9] who measured the partition coefficient of *n*-hexane into egg lecithin at 0° C.

As the mol fraction of *n*-hexane in the bilayer is known, we may calculate how much volume it occupies. If we assume that the partial molal volume of *n*-hexane in dioleoyl phosphatidylcholine is the same as that of hexane in hexane, i.e. 132 cm^3/mol [13], then the maximal volume occupied by hexane in our experiments is 8.3 cm^3/mol. The volume of the hydrocarbon region of dioleoyl phosphatidylcholine was estimated to be 432 cm^3/mol [14] so that the volume fraction occupied by *n*-hexane in the bilayer is 0.019 at the highest hexane concentrations. Similar results are found with egg lecithin.

Fig. 1 shows the partition coefficient K of *n*-hexane between a 0.1 M NaCl solution (which shall henceforth be called water) and dioleoyl phosphatidylcholine dispersion as a function of temperature. Over the temperature range studied (0—55° C), the graph of K vs. T decreases linearly (within experimental error). The data in Fig. 1 can be fit to the least square linear equation

$$K_{DP} = 138936 - 362.5(T + 273);\ r^2 = 0.98 \qquad (0 \leqslant T \leqslant 55) \qquad (2)$$

The partition coefficient of *n*-hexane from water into egg lecithin dispersions decreases linearly (within experimental error) with temperature as seen in Fig. 2. The data in Fig. 2 can be fit to the least square linear equation

$$K_{EL} = 321376 - 853.1(T + 273);\ r^2 = 0.98 \qquad (0 \leqslant T \leqslant 50) \qquad (3)$$

The unitary free energy of transfer, $\overline{\Delta G}$ may be calculated from Eqns. 2 and 3 using the equation

$$\overline{\Delta G} = -RT \ln K = \overline{\Delta H} - T\overline{\Delta S} \qquad (4)$$

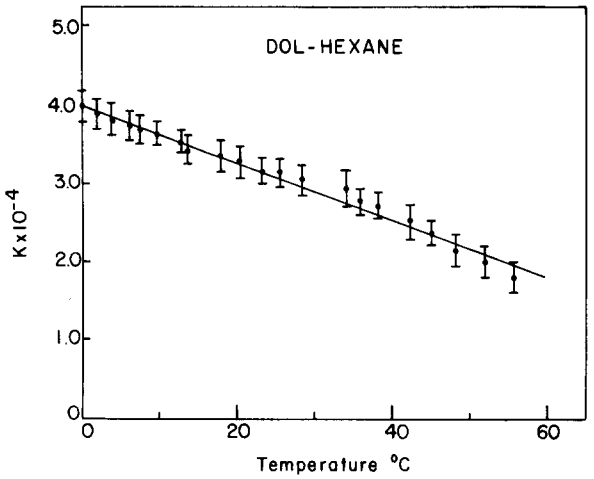

Fig. 1. The partition coefficient of *n*-hexane from water to dioleoyl phosphatidylcholine (DOL) as a function of temperature. The solid line is a least square fit to the data and is given by Eqn. 2 in the text.

The partial molal enthalpy of transfer, $\overline{\Delta H}$ and entropy of transfer $\overline{\Delta S}$ are obtained from Eqn. 4, i.e.

$$\overline{\Delta H} = -R\left(\frac{\partial \ln K}{\partial 1/T}\right) \tag{5}$$

$$\overline{\Delta S} = \frac{\overline{\Delta H} - \overline{\Delta G}}{T} \tag{6}$$

The free energy of transfer as calculated in Eqn. 4 is on a mol fraction basis. That is, X_L is the number of mol of *n*-hexane/number of mol of *n*-hexane +

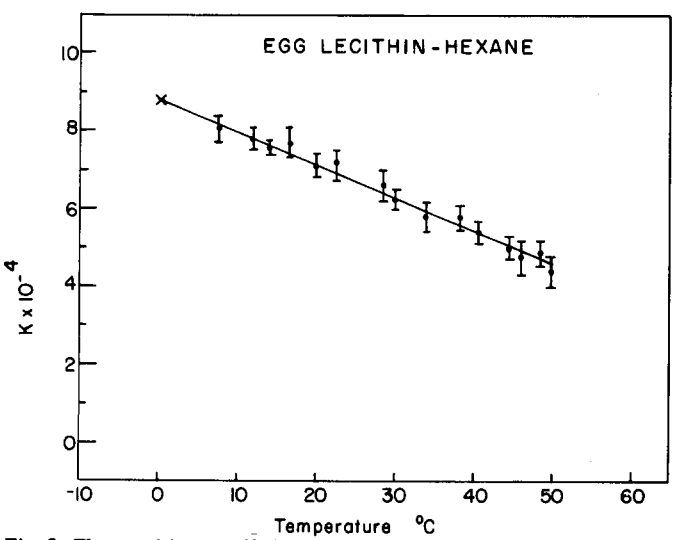

Fig. 2. The partition coefficient of *n*-hexane from water to egg lecithin as a function of temperature. The point indicated by (X) was data obtained from ref. 9. The straight line is a least square fit to the data and is given by Eqn. 3 in the text.

number of mol of dioleoyl phosphatidylcholine. However, as dioleoyl phosphatidylcholine (and egg lecithin) have 2 mol of acyl chains per mol, we could also calculate $\overline{\Delta G}$ on a mol fraction per acyl chain basis. This will change Eqn. 4 to read

$$\overline{\Delta G} = -RT \ln K + RT \ln 2 \quad (7)$$

This has the effect of increasing $\overline{\Delta G}$ by a constant factor at a given temperature. At 25° C this term contributes 410 cal/mol.

The reason for wanting to do this operation is to facilitate the comparison between phospholipids with two acyl chains per mol and organic liquids that have only one. Table I gives the thermodynamic transfer parameters of *n*-hexane into dioleoyl phosphatidylcholine, egg lecithin and a bulk hydrocarbon solvent at 25° C. As is seen, the use of Eqn. 7 makes the bilayer look even more unlike bulk hydrocarbon than if we use Eqn. 4. Thus to minimize this effect, we will use the values calculated on a molar basis keeping in mind, however, that any conclusions that we arrive at would be even more dramatically demonstrated.

Upon comparing the results in Table I and Figs. 1 and 2, it is noticed that the partition coefficient of *n*-hexane into egg lecithin is about twice what it is in dioleoyl phosphatidylcholine over the entire temperature range. The free energy of transfer of *n*-hexane into dioleoyl phosphatidylcholine and egg lecithin is —6.18 and —6.65 kcal/mol, respectively. Both of these values are considerably below that of a hydrocarbon solvent. The difference in free energy between different hydrocarbon solvents will be small compared to the total free energy of transfer [12]. The entropy of transfer of *n*-hexane from water into dioleoyl phosphatidylcholine and egg lecithin is 15.0 and 14.8 cal/

TABLE I

THERMODYNAMIC TRANSFER PARAMETERS ON *n*-HEXANE FROM WATER TO VARIOUS SOLVENTS AT 25°C

Solvent	$\overline{\Delta G}$ (kcal/mol)	$\overline{\Delta H}$ (kcal/mol)	$\overline{\Delta S}$ (cal/mol degree)
Bulk hydrocarbon	—7.74 [a]	0.0 [b]	26.0
Dioleoyl phosphatidylcholine per mol)	—6.18 ± 0.04	—1.7	15.0
Dioleoyl phosphatidylcholine (per acyl chain)	—5.77 ± 0.04	—1.7	13.8
Egg lecithin (per mol)	—6.65 ± 0.04	—2.2	14.8
Egg lecithin (per acyl chain)	—6.24 ± 0.04	—2.2	13.5
Dioleoyl phosphatidylcholine -cholesterol (2 : 1)	—5.92 ± 0.04		
Dioleoyl phosphatidylcholine -cholesterol (1 : 1)	—5.82 ± 0.04		
Egg lecithin-cholesterol (1 : 1)	—6.09 ± 0.04		
n-Octanol	—5.42 [c]		
Sodium dodecylsulfate (SDS)	—6.56 [d]	—2.1	15.0

[a] Ref. 12.
[b] Ref. 24.
[c] Ref. 25.
[d] Date extrapolated from ref. 26.

mol degree, respectively. Both values are considerably smaller than 26 cal/mol degree which is the entropy of transfer of *n*-hexane from water to *n*-hexane. The enthalpies of transfer of *n*-hexane going from water into bilayers is significantly more negative than it going into a hydrocarbon liquid.

The decrease in partition coefficient with increasing temperature implies that the mol fraction of *n*-hexane in the bilayer is decreasing faster than the mol fraction of *n*-hexane in water decreases.

Dioleoyl phosphatidylcholine, egg lecithin-cholesterol bilayers

The incorporation of cholesterol into dispersions of dioleoyl phosphatidylcholine and egg lecithin decreases the partition coefficient in comparison to the pure lipid dispersions. For dispersions of dioleoyl phosphatidylcholine-cholesterol at mol fractions 2 : 1 and 1 : 1, the partition coefficient decreased a factor of 1.5 and 1.8, respectively, at 25° C, relative to a cholesterol-free dioleoyl phosphatidylcholine dispersion. The incorporation of cholesterol into egg lecithin at a 1 : 1 mol ratio decreased the partition coefficient by a factor of about 2.5 at 25° C.

It is noteworthy to mention that the temperature dependence of the partition coefficient was also measured for the above bilayers. For an individual sample, the partition coefficient always decreased with temperature; however, the change over a 35° C temperature range (15—50° C) was relatively small. Because of this, the scatter in the data does not make the enthalpy and entropy of transfer very meaningful numbers.

Discussion

The method we chose to examine the solvent properties of lipid bilayers was to directly measure the partition coefficient. By directly measuring the partition coefficient, we do not have to invoke ad hoc assumptions about the nature of the solvent, position of the probe, and the interpretation of spectra shapes [15].

The reasons for choosing *n*-hexane as the solute are: (1) It is an alkane whose thermodynamic transfer parameters have been experimentally determined [16]. (2) Being an alkane, it cannot form covalent or hydrogen bonds with the solvent or water. Consequently it must interact with these phases through London and/or dipole-induced dipole interactions [13]. (3) It is a relatively small molecule with respect to the volume of the bilayer so that extreme steric restrictions that a larger non-polar molecule may experience are reduced. (4) It has a very large partition coefficient in bilayers above their transition temperature ($\sim 10^4$) so that it will be located "deep" in the bilayer. (5) As a hydrocarbon, it may undergo many of the same conformations that the acyl chains of a bilayer do.

The reasons for choosing dioleoyl phosphatidylcholine and egg lecithin as the solvent are: (1) They are chemically well characterized [17,18]. (2) Their interactions with cholesterol are well studied [19,20]. (3) They are both above their transition temperatures in the temperature ranges investigated (0—55° C) [21].

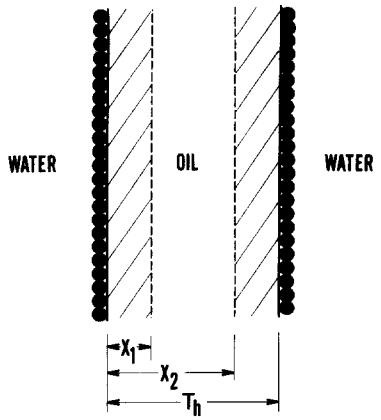

Fig. 3. A schematic model for a lipid bilayer. The regions, X_1, and T_h—X_2 of undefined length are assumed to be totally unavailable for partition of *n*-hexane. The middle region, of length T_h—X_1, designated as oil is to be considered a bulk hydrocarbon liquid.

The solvent properties of dioleoyl phosphatidylcholine, egg lecithin bilayers

We are now in a position to see if dioleoyl phosphatidylcholine and egg lecithin bilayers may be treated as a bulk hydrocarbon solvent. The partition coefficient that we measured is the average value of the partition coefficient [5]. In bilayers, unlike bulk organic liquids, it is likely that the partition coefficient is a function of position in the bilayer [5]. This positional dependence will arise primarily for two reasons. The first is that near the polar head group and a few methylene groups down the chain the bilayer has a more hydrophilic environment than near the center [22]. The second is that the first 6 or 7 methylene groups from the polar head group are relatively ordered in comparison to the remaining 11 or 12. For this reason the entropy of transfer in these regions would be considerably lower than it would be for those regions near the center of the bilayer, which have a much greater chain mobility [23].

Hence we developed a model that incorporates the following ideas. We divide the bilayer into three distinct regions (see Fig. 3). The regions between $0 \leqslant X \leqslant X_1$ and $X_2 \leqslant X \leqslant T_h$ are assumed to be completely unavailable for partition for the reasons given above. The region X_2—X_1 we will assume to be a bulk hydrocarbon liquid. We may then calculate what volume of the bilayer would be available for partition under these conditions. The values for K_{DP}, K_{EL} and K_{HC} are known (Table I) so we may write

$$K_{BL} = \int_0^V K_{HC}\, dv/V \tag{8}$$

where V = volume of bilayer. K_{HC} = partition coefficient of *n*-hexane into bulk hydrocarbon, K_{BL} = partition coefficient of *n*-hexane into a bilayer.

If the partition coefficient of *n*-hexane varies only in the direction perpendicular to the plane [5] of the membrane then Eqn. 7 becomes

$$K_{DP} = A \int_0^{x_1} K_{HC}\, dx + \int_{x_1}^{x_2} K_{HC}\, dx + \int_0^{Th} K_{HC}\, dx \Big/ A\, T_h \tag{9}$$

where A = area of bilayer, T_h = thickness of the bilayer, and x_1 and x_2. The thickness of X_1 and X_2, respectively.

The first and last terms on the right hand of Eqn. 9 are zero by virtue of the assumptions, so that Eqn. 9 reduces to

$$K_{BL} = \frac{A(X_2 - X_1)}{AT_h} K_{HC} = \frac{V_{eff}}{V} K_{HC} \tag{10}$$

The values of K_{DP} and K_{EL}, at 25°C, as calculated from Eqns. 2 and 3 give an effective volume, V_{EFF}, of 7.5 and 16% for dioleoyl phosphatidylcholine and egg lecithin, respectively. That is, if the model were correct, a dioleoyl phosphatidylcholine bilayer would have only 7.5% of its volume available for partition and an egg lecithin bilayer would have about twice as much. In view of the NMR data for dioleoyl phosphatidylcholine which shows that about 10 of the 17 methylene groups are "quite fluid" [23] (similarly for egg lecithin), we assume that our model must be incorrect. This implies that our original assumption that the bilayer resembles a bulk hydrocarbon liquid must be incorrect, at least for a molecule like hexane.

By making the above statement we do not mean that the physical laws of solubility governing alkane interactions with bilayers will be different for bilayers than hydrocarbon solvents, only that the interaction energies will be different in these two systems.

Over the entire temperature range investigated (0—50°C), the partition coefficient of *n*-hexane into egg lecithin is approximately twice what it is in dioleoyl phosphatidylcholine. Two possible reasons may account for the differences. The first being that the effective volume of egg lecithin for the partition of *n*-hexane into it may be about twice as large as that for dioleoyl phosphatidylcholine as seen from Eqn. 10. The heterogeneous chain distribution of egg lecithin as compared to the homogeneous chain distribution of dioleoyl phosphatidylcholine could account, in part, for the differences. Each molecule of egg lecithin has a saturated and unsaturated chain generally of different lengths [18]. The differences in length between adjacent chains and between opposing monolayers may give rise to a larger effective volume than a lipid whose acyl chains are all of the same length. Another factor that could contribute to the higher value of K for egg lecithin and dioleoyl phosphatidylcholine is the greater enthalpy of transfer (Table I). As will be discussed later, this may be due to the presence of a saturated chain.

The question then arises as to what is a good model for the interior of a bilayer? The answer, in part, can be seen in Table I. Upon comparing the thermodynamic transfer values of *n*-hexane from water into dioleoyl phosphatidylcholine and egg lecithin with those extrapolated for *n*-hexane from the data of Wishnia [26] into SDS micelles, we find that they are remarkably similar to each other and both considerably different than either a bulk hydrocarbon solvent or *n*-octanol. Wishnia's data was interpreted by stating the chains in a micelle are more constrained than those in an organic liquid as a result of them being confined to an interface [12]. We feel that a similar effect is seen with dioleoyl phosphatidylcholine and egg lecithin. Although the similarity between SDS and dioleoyl phosphatidylcholine (egg lecithin) may be fortuitous in view that they have different chain lengths, degrees of saturation

and polar head groups, it is noteworthy to point out that SDS has about 100 molecules per micelle and regions that may be two molecules thick [27,18]. The similarities between SDS micelles and bilayers may be why some proteins that are extracted with detergents are still functional.

Quite naturally the incorporation of cholesterol makes the bilayer even more unlike a bulk hydrocarbon liquid. The partition coefficient of *n*-hexane mol basis) from water to various solvents goes in the order

$$K_{HC} > K_{EL} \approx K_{SDS} \gtrsim K_{DP} > K_{DPC} > K_{Oct}$$

Thermodynamic transfer parameters of n-hexane from a bulk hydrocarbon to a bilayer

From the data collected in Table I we are able to calculate the transfer parameters of *n*-hexane from *n*-hexane to a bilayer. Moreover, as previously stated for bulk hydrocarbons, the free energy of transfer of an alkane to a hydrocarbon solvent is not very dependent on the bulk organic solvent that it partitions into, i.e. the difference in free energies of transfer in different solvents is small when compared to the total free energy of transfer [12]. Table II shows the thermodynamic transfer parameters at 25°C of *n*-hexane into various bilayers as well as the enthalpy and entropy of fusion of *n*-hexane in going from its solid state to its isotropic liquid state. From these data it is observed that energetically *n*-hexane would prefer to be associated with itself than with a lipid bilayer. Enthalpically the *n*-hexane would prefer to be in a lipid bilayer (or SDS micelle) whereas entropically it would much prefer to be in an isotropic liquid.

Entropically this result is not surprising as it has been shown that the hydrocarbon tails of bilayers are more restricted in their motion than organic liquids [21,23]. Upon comparison of the entropy of fusion, ΔS^f, (in going from the liquid to the solid state) with the entropy of transfer of *n*-hexane from itself to either dioleoyl phosphatidylcholine or egg lecithin, we find the latter is about 60% of the entropy of fusion. Although we have not been able to get reliable

TABLE II

THERMODYNAMIC TRANSFER PARAMETERS OF *n*-HEXANE FROM *n*-HEXANE TO VARIOUS SOLVENTS AT 25°C

Solvent	$\overline{\Delta G}$ (kcal/mol)	$\overline{\Delta H}$ (kcal/mol)	$\overline{\Delta S}$ (cal/mol degree)	ΔH^f (kcal/mol)	ΔS^f (cal/mol degree)
Dioleoyl phosphatidylcholine	+1.56	−1.7	−11.0	3.12	17.6
Egg lecithin	+1.09	−2.2	−11.0		
Dioleoyl phosphatidylcholine -cholesterol (2 : 1)	+1.82				
Dioleoyl phosphatidylcholine -cholesterol (1 : 1)	+1.92				
Egg lecithin-cholesterol (1 : 1)	+1.65				
n-Hexane [a]				3.12	17.6

[a] Ref. 29.

enthalpy data for the phosphatidylcholine-cholesterol bilayers it is likely that the reason that cholesterol reduces the partition coefficient is because the chains are more restricted and consequently the entropy of transfer would be reduced.

For the above bilayers the enthalpy of transfer of *n*-hexane from itself into a bilayer is about half its enthalpy of fusion (in going from a liquid to a solid). Thus the insertion of *n*-hexane from itself into a bilayer has the effect of "half freezing" the molecule.

The increase in enthalpy that *n*-hexane gains upon being transferred from a bulk hydrocarbon liquid to a bilayer at 25° C can be rationalized in the following manner: First the great decrease in entropy that accompanies the transfer implies that the number of possible conformations that *n*-hexane will have in a bilayer will be less than those it will have in an organic liquid. Once inside the bilayer the *n*-hexane molecule will try to maximize the number and the strength of the bonds it can make with the acyl chains. This may be accomplished by aligning itself, on the average, parallel to the acyl chains between adjacent lipid molecules.

It might be argued that the increase in enthalpy could in part be due to the presence of double bonds in dioleoyl phosphatidylcholine and egg lecithin as this region would have a higher polarizibility than the methylene groups of the chains. Consequently a higher induced dipole-induced dipole interaction of *n*-hexane in this bilayer than an organic liquid might be expected. However, the enthalpy of transfer of *n*-hexane into dimyristoyl phosphatidylcholine, a saturated lipid, at 25° C, is similar to that found for egg lecithin (Simon, S.A., Stone, W.L. and Busto-Latorre, P., unpublished results).

Another region of the membrane that the *n*-hexane could be located would be at the geometric center of the bilayer. However, White [30] pointed out that this region has a high density of methyl groups and as these groups have a lower polarizibility than methylene groups (implying a lower induced dipole-induced dipole interaction), it is unlikely that the molecule is located in this region.

Thus by virtue of the above reasoning, we conclude that at 25° C, for the mol fractions studied, *n*-hexane is, on the average, aligned parallel to the acyl chains.

Conclusions

Throughout this paper we have compared the thermodynamic transfer parameters of *n*-hexane into lipid bilayers to those of micelles and bulk isotropic liquids. We have noted the similarities and differences between these systems and conclude that for a molecule the size and shape of *n*-hexane: (1) The bare lipid bilayer cannot simply be treated as a bulk hydrocarbon liquid. (2) At the mol fractions studied, *n*-hexane is aligned, on the average, parallel to the acyl chains. (3) The addition of cholesterol to egg lecithin and dioleoyl phosphatidylcholine bilayers reduces the partition coefficient, by at most, a factor of 2.5 at 1 : 1 mol ratios. (4) The thermodynamic transfer parameters are approximately the same for SDS, dioleoyl phosphatidylcholine and egg lecithin.

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References

1 Overton, E. (1902) Pflueger Arch. 92, 115—280
2 Seeman, P. (1972) Pharm. Rev. 24, 583—655
3 Collander, R. (1954) Physiol. Plant 7, 420—445
4 Diamond, J.M. and Wright, E.M. (1969) Ann. Rev. Physiol. 31, 581—646
5 Diamond, J.M. and Katz, Y. (1974) J. Membrane Biol. 17, 121—154
6 Cohen, B. (1975) J. Membrane Biol. 20, 205—234
7 Wolosin, J.M. and Ginsburg, H. (1975) Biochim. Biophys. Acta 389, 20—33
8 Wishnia, A. and Pinder, Jr., T.W. (1966) Biochemistry 5, 1534—1542
9 Stone, W.L. (1975) J. Biol. Chem. 250, 4386—4390
10 Bartlett, G.R. (1959) J. Biol. Chem. 234, 466—468
11 Bartlett, G.R. (1959) J. Biol. Chem. 234, 469—471
12 Tanford, C. (1973) The Hydrophobic Effect, J. Wiley and Sons, New York
13 Hildebrand, J.H. and Scott, R.L. (1964) The Solubility of Nonelectrolytes, Dover Publications, New York
14 Tijima, K. and Gershfield, N.L. (1975) Advances in Chemistry Series. Number 144, Am. Chem. Soc., Washington, D.C.
15 Dix, J.A., Diamond, J.M. and Kivelson, D. (1974) Proc. Natl. Acad. Sci. U.S. 71, 474—478
16 Gill, S.J. and Wadso, I. (1976) Proc. Natl. Acad. Sci. U.S. 73, 2955—2958
17 Barton, P.G. and Gunnstone, F.D. (1975) J. Biol. Chem. 250, 4470—4476
18 Graham, D.E. and Lea, E.J.A. (1972) Biochim. Biophys. Acta 274, 286—293
19 Lecuyer, H. and Dervichian, D.G. (1969) J. Mol. Biol. 45, 39—57
20 Engelman, D.M. and Rothman, J.R. (1972) J. Biol. Chem. 247, 3694—3697
21 Phillips, M.C., Williams, R.M. and Chapman, D. (1969) Chem. Phys. Lipids 3, 234—254
22 Griffith, O.H., Dehlinger, P.J. and Van, S.P. (1974) J. Membrane Biol. 15, 159—171
23 Lee, A.G., Birdsall, N.L.M. and Metcalfe, J.C. (1971) in Methods in Membrane Biology (Korn, E.D., ed.), Vol. 2, pp. 2—108, Plenum Press, New York
24 Gill, S.J., Nichols, N.F. and Wadso, I. (1976) J. Chem. Thermodyn. 8, 445—452
25 Hansch, C., Quinlan, T.E. and Lawrence, G.L. (1968) J. Org. Chem. 33, 347—350
26 Wishnia, A. (1963) J. Chem. Phys. 67, 2079—2093
27 Tanford, C. (1974) J. Phys. Chem. 78, 2469—2479
28 Israelachvili, J.N., Mitchell, D.J. and Ninham, B.W. (1976) J. Chem. Soc. Faraday Trans. II 72, 1525—1578
29 Handbook of Chemistry and Physics (1975) 55th edn., (Weast, R.C., ed.), CRC Press, Cleveland, Ohio
30 White, S.H. (1976) Nature 262, 421—422