Transport of Biological Lipophiles: Effect of Lipophile Structure

Henry J. Pownall,* Diane L. Hickson, and Louis C. Smith

Contribution from the Baylor College of Medicine and The Methodist Hospital, Houston, Texas 77030. *Received August 30, 1982*

Abstract: A systematic study of the structural determinants involved in the spontaneous transfer of molecules between single bilayer vesicles of phosphatidylcholine is reported. All of the molecules studied contain a pyrenyl moiety whose excimer fluorescence provides a direct measure of the changes in its microscopic concentration. These compounds include pyrenyl alkanes, alcohols, carboxylic acids, and their methyl esters. In each group, transfer between vesicles occurs via the intervening aqueous phase. The rate of transfer is a function of both the hydrophobicity (chain length) and the hydrophilicity (polar or nonpolar) of the transferred species. The rates of transfer can be expressed in terms of a free energy of activation, $\Delta G^{\ddagger}$, which is calculated from absolute rate theory. A good correlation exists between $\Delta G^{\ddagger}$ and ΔG_t, the free energy of molecular transfer from a hydrophobic environment to the aqueous phase. The rate of transfer increased both with decreasing chain length in a given homologous series and with the polarity of the substituents if the number of methylene units is constant. The incremental $\Delta G^{\ddagger}$ for the polar compounds was $\simeq$740 cal/methylene unit, whereas the corresponding value for the alkyl pyrenes is $\sim$900 cal/methylene unit. These values are similar to the reported ΔG_t per methylene unit calculated from equilibrium measurements. The $\Delta G^{\ddagger}$ per methylene unit of the polar compounds reflected changes in the $\Delta H^{\ddagger}$ since $\Delta S^{\ddagger}$ was independent of chain length. By contrast, the alkyl pyrenes exhibited very large changes in $\Delta H^{\ddagger}$ with increasing chain length ($\simeq$2 kcal/methylene unit) that are, in part, compensated by changes in $\Delta S^{\ddagger}$. As a consequence, only a small difference in the contribution of each methylene to $\Delta G^{\ddagger}$ of transfer of alkanes and amphiphiles is predicted.

The mechanism of transport of molecules in vivo is an important but poorly defined aspect of our overall understanding of how various substances are utilized by the body. The transport mechanisms that are of interest here are those that involve amphiphilic and hydrophobic molecules; this includes the normal components of blood and tissue such as lipids, steroid hormones, and vitamins and other agents such as anesthetics, pesticides, carcinogens, and certain drugs. A number of mechanisms have been proposed for transport between lipid-rich environments of cell membrane and plasma lipoproteins. These include (a) transport by specific carriers that are usually proteins, (b) fusion with transport occurring at the point of contact between the donor and acceptor surfaces, and (c) passive transport via the intervening aqueous phase.[1-3] Specific examples of the first mechanism include fatty acid transport by albumin and the transport of cholesteryl esters and phospholipids between membranes by specific proteins isolated from plasma or cytoplasm from various tissues.[2-7] A fusion mechanism has been proposed for the transfer of lipids between both cells and plasma lipoproteins or lipid vesicles, and a number of in vitro experiments have demonstrated this in model systems.[8,9] Finally, the passive transfer of molecules through the aqueous space between two compartments has emerged as an important mechanism for the transport of other kinds of molecules.[10-17] Its importance to the transfer of sparingly soluble molecules is now recognized. Herein we present a systematic examination of some of the structural determinants that control the rates of passive transfer of lipid-soluble molecules.

Our experimental approach has been to prepare pyrenyl analogues of various molecular species and to measure their rates of transfer by a fluorescence technique. Pyrene is a hydrocarbon that forms fluorescent excited dimers (excimers) at relatively low concentrations. The number of excimers increases linearly with the microscopic pyrene concentraton, so that the relative intensity of excimer fluorescence may be used to follow changes in the pyrene concentration.[12-15] This correlation is also valid for many derivatives of pyrene, provided there is no effect on the local electronic structure of the pyrene moiety. Figure 1 contains a schematic representation of how the transfer of a pyrenyl compound effects a reduction in its microscopic concentration and, consequently, a decrease in its excimer fluorescence intensity. Since the excimer fluorescence is directly proportional to the microscopic concentration, the rate of change of the latter is identical with that of the former.

Experimental Section

The pyrenyl analogues of saturated fatty acids, alcohols, methyl esters, and alkanes of various hydrocarbon chain length (Figure 2) were either obtained from Molecular Probes (Plano, TX) or synthesized according to published procedures. Very briefly, the pyrenyl alkanes and fatty acids were formed by a Wittig reaction of pyrene-3-carboxaldehyde and the appropriate phosphorus ylid followed by a catalytic reduction.[18] Purification of the alkanes and acids, respectively, was achieved by elution over silica gel in hexane and hexane–ethyl acetate (6:4). The pyrenyl methyl esters were formed by refluxing the corresponding fatty acid in methanolic BF_3 for 30 min followed by purification over silica gel eluted with hexane–ethyl acetate (7:3). All pyrenyl compounds gave a single fluorescent spot in several solvent systems and had satisfactory mass spectra. *sn*-1-Palmitoyl-2-palmitoleoylphosphatidylcholine (PPOPC) was synthesized from 1-palmitoyllysophosphatidylcholine by the method of Mason et al.[19] and purified according to Patel et al.[20]

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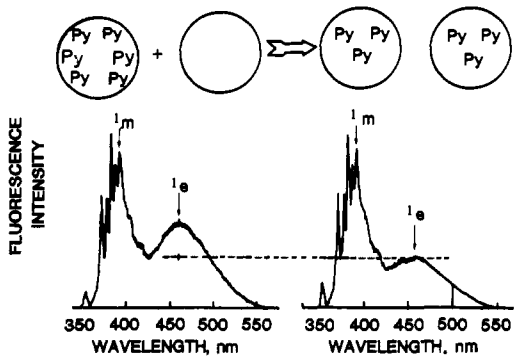

Figure 1. Schematic representation of the spectroscopic consequences of pyrene transfer from one compartment to another.

FLUORESCENT PROBES OF LIPID TRANSFER RATES

HYDROCARBONS–	PYRENE ≡ ⟨H⟩ ≡ Py–H
	Py–R R≡CH_3–$(CH_2)_n$–
FATTY ACIDS	Py–$(CH_2)_n$–C(=O)–OH
ALCOHOL	Py–$(CH_2)_n$–OH
METHYL ESTER	Py–$(CH_2)_n$–C(=O)–O–CH_3

n = Number of methylene units

Figure 2. Pyrenyl compounds used in this study.

Methods. Fluorescent Labeling. Single bilayer vesicles of PPOPC having a mean radius of 150 Å were prepared according to Barenholz et al.[21] Unless otherwise specified, a buffer of 100 mM NaCl, 10 mM Tris, pH 7.4, 0.01% EDTA, and 0.01% NaN_3 was used throughout. The donor species in this study were labeled vesicles. A small amount (2–4 mol %) of the fluorophore was added to PPOPC in chloroform, the solvent was removed under vacuum, and the vesicles were prepared as usual.[21]

The fast kinetics were conducted on a stopped-flow unit as described by Doody et al.[15] Transfer rates that were on the order of minutes or longer were measured on an SLM photon counting fluorometer operated in the T-Y format. Between 3 and 10 decay curves were collected and their rate constants averaged to obtain the given rate constants. All experiments were conducted in a >10-fold excess of acceptor species to maximize the signal change and to reduce the contribution of the reverse transfer process. The acceptor vesicles were identical with the donor but without probe.

Results

Fluorescence Spectra of Pyrene Derivatives in PPOPC Single Bilayer Vesicles. The fluorescence spectra of the pyrene derivatives of this study are similar to that of pyrene in single bilayer vesicles of phosphatidylcholine. There were no significant differences in the fine structure of the monomer spectra of the different pyrenyl derivatives. At low ratios of pyrene to PPOPC, the fluorescence spectrum is composed only of monomer fluorescence, but at higher concentrations, an excimer fluorescence band centered at 475 nm appears. The relative intensity of the excimer fluorescence of each pyrenyl derivative increases with its microscopic concentration in PPOPC. This may be expressed as the ratio of the excimer

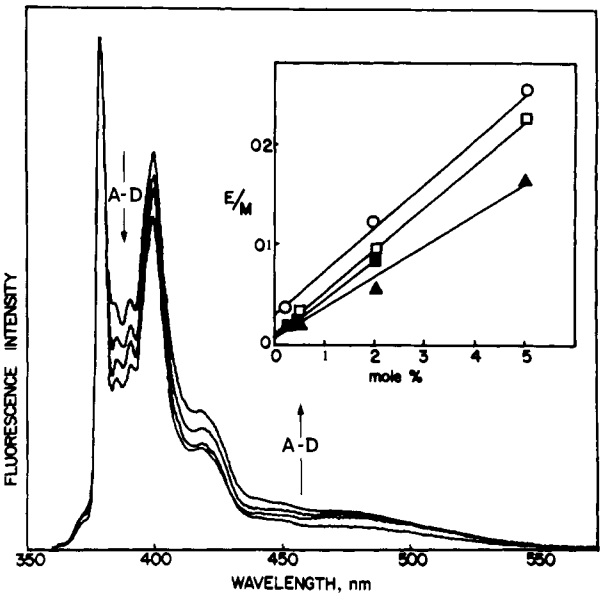

Figure 3. Fluorescence of 3-octylpyrene in PPOPC single bilayer vesicles. A, B, C, and D, respectively, equal 0.25, 0.5, 2, and 5 mol %. Inset: Plot of the ratio of excimer to monomer fluorescence intensity vs. mole percent of the pyrenyl compound in single bilayer vesicles of PPOPC. All four derivatives contain 10 carbons in the side chain attached to pyrene side chain; other derivatives in each class gave a similar correlation. Fatty acid (■); alkane (○); alcohol (▲); methyl ester (□).

to monomer fluorescence intensities as illustrated in Figure 3 for a series of derivatives having the same number of methylene units. All four compounds gave a linear increase in the excimer/monomer ratio as a function of mole percent in the PPOPC vesicle. Therefore, the excimer fluorescence may be used to monitor the changes in the microscopic concentration of the pyrene derivatives. The slopes of the curves increased in the order alcohol < acid ≃ ester < alkane.

In a series of experiments, the rates and activation energies for the transfer of each pyrenyl analogue between PPOPC single bilayer vesicles were determined. In all cases the transfer was first order and independent of concentration and donor to acceptor ratio. For example, at 25 °C the average rate constant for the transfer of octylpyrene from labeled (5×10^{-6} M) to unlabeled PPOPC vesicles was (1.24 ± 0.04) × 10 with acceptor to donor vesicle ratios of 10, 20, 50, and 100. In other experiments (unpublished data) in which different lipid acceptors were used, no differences in the transfer rate were observed. Some representative rate data are shown in Figure 4. Figure 4A shows that with a given alkyl pyrene, 1-(3-pyrenyl)decane, kinetics of transfer are first order and the rate of transfer increases with increasing temperature. By contrast, at constant temperature the rate of transfer decreases with increasing chain length as shown by the data of Figure 4B. These effects are compared in Figure 5, which contains Arrhenius plots of the transfer of all of the alkyl pyrenes of this study. In addition to the effect of temperature and chain length on the transfer rate, the change in the slopes of the plots with chain length demonstrates that the activation energy for transfer increased with the chain length. Similar effects of chain length and temperature on the rate and activation energy for transfer were observed for all of the pyrenyl derivatives of this study (Table I). Within a given homologous series, the incremental change in the activation energy with chain length (i.e., kilocalories per CH_2) was constant. These were calculated from the data of Figure 5 and are given in Table I. The activation energies for the alcohols at pH 7.4 and for the esters and acids at acidic and basic pHs were similar and were 850 ± 40 cal/CH_2. By contrast, the incremental activation energy for the alkyl pyrenes were nearly twice as large (1950 ± 80 cal/CH_2). Further inspection of Figure 6 reveals that at a given chain length, the activation energy for transfer increased in the order ester, pH 2.8,

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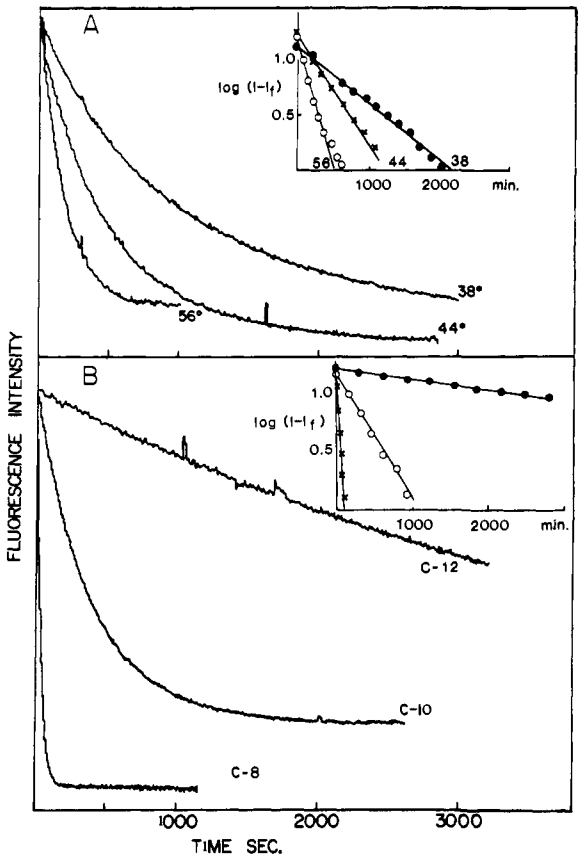

Figure 4. Decay of excimer fluorescence as a function of time. (A) 1-(3-pyrenyl)decane at the three indicated temperatures. (B) Three different pyrenyl alkanes (the number of carbon atoms in the side chain is shown with the curve) at one temperature, 45 °C: (●) C-12; (○) C-10; (×) C-8). All curves were obtained by mixing labeled single bilayer vesicles of PPOPC with an excess of those having no label. The insert contains the first-order plot of the same data where I and I_f are the observed and final intensities, respectively.

< acid, pH 9.0, < alcohol < acid, pH 2.8, < ester, pH 7.4. In general, the rate of transfer decreased in the same order (Table I).

From the rate constant at 25 °C and the activation energies we have used absolute rate theory to calculate the transition-state parameters for the transfer of the various pyrene derivatives. The values for the enthalpy ($\Delta H^{\ddagger}$), entropy ($\Delta S^{\ddagger}$), and free energy of activation ($\Delta G^{\ddagger}$) are given in Table II. The changes in these parameters with chain length are plotted in Figure 7. The activation parameters for the alcohols, acids, and esters were similar. As a function of chain length $\Delta S^{\ddagger}$ changed very little, whereas the incremental increases in $\Delta G^{\ddagger}$ and $\Delta H^{\ddagger}$ were necessarily similar to those observed for the incremental activation energies. By contrast, the behavior of the alkyl pyrenes was, in some important respects, different. The incremental change in $\Delta H^{\ddagger}$ was, of course, identical with that given above for the activation energy; i.e., it was approximately double that of the other pyrene derivatives. However, unlike the other derivatives, the magnitude and sign of the incremental change in $T\Delta S^{\ddagger}$ was such that a substantial portion of the contribution of changes in $\Delta H^{\ddagger}$ to $\Delta G^{\ddagger}$ that appear with increasing chain length was offset by the $T\Delta S^{\ddagger}$ component. As a consequence, the incremental $\Delta G^{\ddagger}$ for the alkyl pyrene series was only slightly larger (900 ± 40 cal/CH_2) than those of the other pyrene derivatives (average = 765 ± 40 cal/CH_2).

Discussion

The kinetics of transfer of the pyrenyl derivatives are consistent with a mechanism involving their rate-limiting desorption from the vesicle into the surrounding aqueous phase followed by diffusion controlled movement to an acceptor vesicle. The experimental criteria for this mechanism given by Charlton et al.[13] are that the process is first order and independent of donor and acceptor concentration and of the identity of the acceptor; our data satisfy all three of these criteria.

The effects of altering the chain length or the functional group on the lipophile transfer rate are also qualitatively consistent with this mechanism. The velocity, v_i^-, for the desorption of the ith component is proportional to the lipid concentration and the mole fraction, X_i, of the desorbing species in the single bilayer vesicle, SBV. This is given by

$$v_i^- = k_i^-[\mathrm{SBV}]X_i \qquad (1)$$

where k_i^- is the rate constant, which is dependent upon the identity of i. [SBV] = [PC]/n where n is the aggregation number.

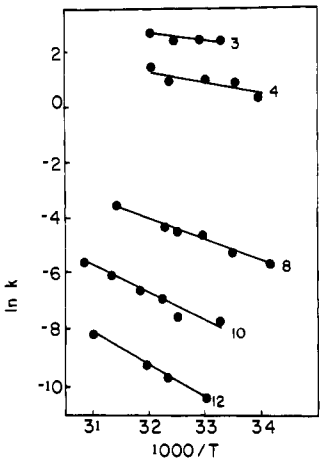

Figure 5. Arrhenius plots of the kinetics of transfer of pyrenyl alkanes between single bilayer vesicles of PPOPC. The numbers adjacent to each line are the number of carbon atoms in the aliphatic chain (i.e., 3 = propyl, etc.).

Table I. Rates and Activation Energies for the Transfer of Pyrenyl Derivatives between PPOPC Single Bilayer Vesicles at 25 °C

derivative	n^b	k, s^{-1}	E_a, kcal	ΔE_a,[c] incremental E_a, kcal/CH_2
alcohol (7.4)[a]	7	3.2	8.0	
	9	0.62	9.7	0.80
	10	0.073	10.5	
	12	0.0061	12.0	
ester (7.4)	6	1.3	9.1	
	8	0.09	10.7	0.83
	9	0.02	11.6	
ester (2.8)	6	5.0	5.2	
	8	0.42	7.0	0.90
	9	0.12	7.9	
acid (9.0)	6	100	5.8	
	8	22	7.4	0.84
	9	4.8	8.2	
	11	0.37	9.8	
acid (2.8)	6	8.8	8.5	
	9	0.16	11.0	0.80
	11	0.018	12.5	
alkanes (7.4)	3	9.5	5.5	
	4	2.0	7.3	2.0
	8	0.006	15	
	10	3×10^{-4}	19.5	
	12	1.7×10^{-5}	23	

[a] Number in parentheses is the pH. [b] n = number of methylene units. [c] Obtained from a plot of E_a vs. n in Figure 6. The value of k at 25 °C was obtained by extrapolation or interpolation of the Arrhenius plots.

Table II. Thermodynamics of the Activated State for the Transfer of Pyrenyl Derivatives between PPOPC Single Bilayer Vesicles at 25 °C[a]

derivative	n[c]	$\Delta H^{\ddagger}$,[d] kcal mol^{-1}	$\Delta\Delta H^{\ddagger}$, kcal mol^{-1} n^{-1}	$\Delta S^{\ddagger}$, eu mol^{-1}	$\Delta\Delta S^{\ddagger}$, eu mol^{-1} n^{-1}	$\Delta G^{\ddagger}$, kcal mol^{-1}	$\Delta\Delta G^{\ddagger}$,[d] kcal mol^{-1} n^{-1}
alcohol (7.4)[b]	7	7.4		31		16.8	
	9	9.1	0.80 (0.036)	29	nil	17.7	
	10	9.9		30		19.0	0.76 (0.11)
	12	11.4		30		20.5	
ester (7.4)	6	8.5		30		17.3	
	8	10.1	0.83 (0.08)	29	nil	18.9	0.80 (0.26)
	9	11.0		29		19.7	
ester (2.8)	6	4.6		40		16.5	
	8	6.4	0.90 (0.011)	39	nil	18.0	0.74 (0.25)
	9	7.3		38		18.7	
acid (9.0)	6	5.2		32		14.7	
	8	6.8	0.84 (0.007)	30	nil	15.6	0.69 (0.18)
	9	7.6		30		16.5	
	11	9.2		30		18.1	
acid (2.8)	7	7.9		28		16.2	
	10	10.4	0.80 (0.049)	27	nil	18.6	0.74 (0.23)
	12	11.9		27		19.9	
alkanes (2.8–9.0)	3	4.9		38		16.1	
	4	6.7	1.98 (0.1)	35	−4	17.0	0.88 (0.46)
	8	14.6		20		20.5	
	10	18.9		11		22.3	
	12	22.7		4		24.0	

[a] Thermodynamic values of the transition state were calculated from $\Delta H^{\ddagger} = E_a - RT$ and $\Delta S^{\ddagger} = 2.303\,R \log [NhX/(RT)]$, where R is the gas constant, N is Avogadro's number, h is Planck's constant, and X = (rate k)/exp $\Delta H/(RT)$]. The free energy of activation is given by $\Delta G^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger}$. [b] Values in parentheses are the pHs at which the experiments were conducted. [c] n = number of methylene units. [d] The differences in the incremental values for $\Delta G^{\ddagger}$ and $\Delta H^{\ddagger}$ are significant according to a simultaneous test procedure which is a posteriori test for differences among a set of regression coefficients.[22] Value in parentheses is the standard error of regression.

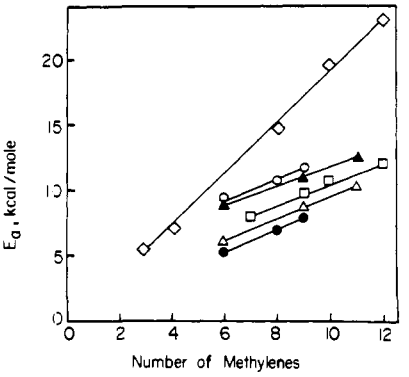

Figure 6. Chain length dependence of the activation energy for molecular transfer of pyrenyl alkanes, pH 2.8–7.4 (◇), alcohols (pH 2.8–7.4) (□), methyl esters (pH 7.4) (○), methyl esters (pH 2.8) (●), fatty acids (pH 9.0) (△), and fatty acids (pH 2.8) (▲).

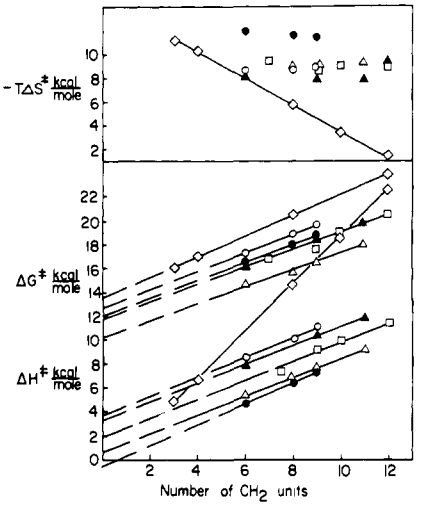

Figure 7. Chain length dependence of the transition-state parameters for molecular transfer of pyrenyl species between single bilayer vesicles of PPOPC. Alkanes (pH 2.7–7.4) (◇); alcohols (pH 2.8–7.4) (□); methyl esters (pH 7.4) (○); methyl esters (pH 2.8) (●); fatty acids (pH 9.0) (△); fatty acids (pH 2.8) (▲).

The velocity, v_i^-, for the association of the ith component with a vesicle is given by

$$v_i^+ = k_i^+[\mathrm{i}][\mathrm{SBV}] \tag{2}$$

where k_i^+ is the rate constant for the association of the ith component with a lipid vesicle. At equilibrium, $v_i^+ = v_i^-$ so that

$$k_i^- = \frac{[\mathrm{i}]}{X_i} k_i^+ \tag{3}$$

and the equilibrium constant is

$$K_{eq} = \frac{k_i^-}{k_i^+} = \frac{[\mathrm{i}]}{X_i} \tag{4}$$

For the case where the ith component is distributed between water and the PC, one can calculate the free energy of transfer:

$$\Delta G_t = -RT \ln [\mathrm{i}]/X_i \tag{5}$$

If we consider the transfer of a lipid molecule from a vesicle composed only of one component ($X_i = 1$), we have

$$\Delta G_t = -RT \ln [\mathrm{i}] \tag{6}$$

where [i], the aqueous concentration of i, is equivalent to the critical micelle concentration (cmc) or solubility of the ith component in water; this form is similar to that given by Tanford for calculating the free energy of micellization.[23]

If the affinity of a lipophile with a vesicle is governed by the

(23) Tanford, C. "The Hydrophobic Effect"; Wiley-Interscience: New York, 1980. Tanford has expressed the free energy of transfer of a monomeric amphiphile to a micelle in terms of the equation $\Delta G_t = RT\,[\ln X_w f_w + m^{-1} \ln (X_{mic} m^{-1})]$ where X_w and X_{mic} are the equilibrium mole fractions of amphiphile in water and micelle, respectively, m is the aggregation number, and f_w is the activity coefficient. We have used $f_w = 1$ for convenience and ignored the second term. Since the value of m in vesicles is about 2000, this assumption seems justified. Note that the ΔG_t that we discuss in the text is for the removal of a monomer from a micelle to water to permit comparison with our transfer data in the same direction. This convention is in the opposite direction to that discussed in most of the literature and by Tanford so that our sign for ΔG_t is opposite to that given above for ΔG_t.

Table III. Head-Group Effects on Activation Parameters[a]

pyrenyl species	$\Delta\Delta G^{\ddagger}_0$(HG), kcal[b]
alcohol	+2.8
ester (7.4)	+1.6
ester (2.8)	+2.5
acid (9.0)	+4.5
acid (2.8)	+2.8
alkane	0

[a] To avoid the uncertainties of extrapolation to zero methylene units, we obtained the head-group contribution from the differences in $\Delta G^{\ddagger}$ at methylene unit number eight of Figure 7. [b] Calculated according to $\Delta\Delta G^{\ddagger}_0(HG) = \Delta G^{\ddagger}(alkane) - \Delta G^{\ddagger}(j)$ where $\Delta G^{\ddagger}$(alkane) and $\Delta G^{\ddagger}(j)$ are the respective free energies of activation of octylpyrene and the polar analogues.

hydrophobic effect, then increasing the chain length would increase the free energy of association and decrease the rate of desorption. If we assume that k_i^+ is independent of chain length,[24,25] eq 3 states that the relative solubility of a lipophile both in water and in a vesicle will govern its distribution between those two compartments and its rate of desorption from the vesicle. This correlation also holds for the effects of a change of the functional group on a homologous series of compounds. The rates of transfer of the more hydrophilic derivatives are faster than those of their less water-soluble analogues. This is consistent with the observations of Doody et al.,[15] who reported that between single bilayer vesicles of dimyristoylphosphatidylcholine, the anion of pyrenylnonanoic acid was transferred faster than the protonated form.

By applying absolute rate theory to our data, we have obtained transition-state parameters, which appear to provide a quantitative theoretical model for our observations (Table II). The variation of $\Delta G^{\ddagger}$ with the number of methylene units correlates very well with the reported contribution of each methylene unit to the free energy of transfer from the hydrophobic to the aqueous phase, ΔG_t.[23] In fact, the slightly higher incremental $\Delta G^{\ddagger}$ per methylene unit of the alkyl pyrenes relative to the ones with a polar group compares favorably with the correspondingly greater ΔG_t per methylene unit obtained from equilibrium measurements of alkanes and amphiphiles, respectively. Furthermore, the effect of a functional group on $\Delta G^{\ddagger}$, obtained from the difference in $\Delta G^{\ddagger}$ for compounds containing eight methylene units in the side chain (the last column of Table III), shows that the free energy of activation for lipophile transfer decreases in the order alkane > ester (7.4) > ester (2.8) ~ acid (2.8) ~ alcohol (2.8–7.4) > acid (9.0). This trend is that predicted from the increase in the aqueous-phase solubility of lipophiles and the decrease in their free energy of transfer from hydrocarbon to water. It is known that both $-CO_2H$ and $-OH$ groups shift the free energy of transfer of an aliphatic moiety by 4–5 kcal,[26] an effect that is probably due to the formation of additional hydrogen bonds in water. By analogy, we suggest that the transfer of the polar pyrene derivatives from the vesicle to the transition state involves the formation of additional hydrogen bonds. This environment could be either bulk water or ordered interfacial water; it is not possible to distinguish the two possibilities on the basis of our results. An additional internal comparison is provided by the acids, which were measured at both acidic and basic pH values. The ionization of a fatty acid is known to greatly increase its aqueous phase solubility and to decrease its free energy of transfer from hydrocarbon to water. In our studies the free energy of activation for the transfer of the ionized form of the fatty acids is always less than that of the same acid in its protonated form. This difference, ~2 kcal, is of the same sign and magnitude as difference in the free energy of transfer of fatty acids from hydrocarbon to water measured under acidic and basic conditions. The hydrogen-bonding capacity of the carboxyl group can be reduced via formation of its methyl ester, and the solubility of the methyl ester is expected to be less than that of the anion but similar to that observed at pH 2.8. Once again, there appears to be a correlation between $\Delta G^{\ddagger}$ and ΔG_t. Values for $\Delta G^{\ddagger}$ for the acid and ester at pH 2.8 are similar and less than that of the anion at pH 9.0. Therefore, there can be little doubt that the activated state in lipophile transfer between phospholipid vesicles is in either the aqueous phase or an interfacial aqueous region near the phospholipid head group that has properties very similar to those of bulk phase water.

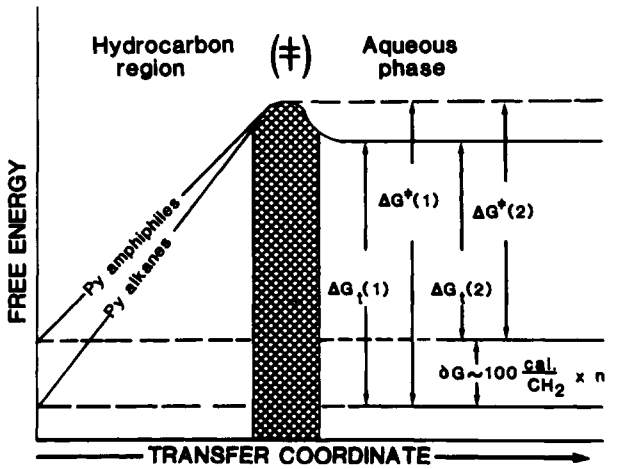

Figure 8. Schematic comparison of the reaction coordinates for the transfer of pyrenyl alkanes and amphiphiles from a lipid bilayer to water. The initial point (zero point of abscissa) is the middle of the bilayer for alkanes but is a region closer to the surface for the amphiphilic compounds. The difference in the slopes of the plot for pyrene alkanes and pyrene amphiphiles from the beginning of the transfer process to the transition state (‡) is exaggerated for the sake of illustration. The numbers 1 and 2 in parentheses refer to the free energy changes for pyrenyl alkanes and amphiphiles, respectively. The scales are not absolute with respect to energy changes nor with respect to differences between the pyrenyl alkanes and the pyrenyl amphiphiles. δG represents the difference between the sum of the incremental ΔG_t or $\Delta G^{\ddagger}$ of the amphiphiles and alkanes.

Clearly, the contribution of $\Delta H^{\ddagger}$ to the incremental $\Delta G^{\ddagger}$ for the alkyl pyrenes is different from that of the amphiphilic pyrenes. The source of this difference is not clear. We speculate that the alkyl pyrenes, which lack a polar group, might localize in the middle of the lipid bilayer where they have additional degrees of freedom or associate with the acyl chains through a process that is thermodynamically similar to cocrystallization. However, the fine structure of the pyrene monomer fluorescence, which can be used as a polarity probe,[27] is nearly the same in all the compounds studied, so that it is not possible to make an unambiguous assignment.

Except for the alkyl pyrenes, the entropy of activation for the transfer of all of the lipophiles tested is independent of chain length and differs very little between pairs of homologues. As a consequence, the variations of $\Delta G^{\ddagger}$ with chain length are similar to those of their respective changes in $\Delta H^{\ddagger}$. By contrast, the changes in $\Delta H^{\ddagger}$ and $T\Delta S^{\ddagger}$ with the chain length of the alkyl pyrenes are much larger. Their effects on $\Delta G^{\ddagger}$, however, are opposite in sign so that the incremental $\Delta G^{\ddagger}$ for the alkyl pyrenes is only slightly greater than those of the other lipophiles. Although compensating

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(26) The contribution of a hydroxyl or carboxyl group to the partitioning of an aliphatic substance between water and hydrocarbon is on the order of 5 kcal.[23] The difference between this value and ours is probably significant and may be attributed to differences between a hydrophobic compartment that is a bulk liquid and one in which a substantial portion of the system is composed of an interfacial region of lipid and water. In the latter the polar groups are transferred into a distinctly different region but in phospholipid vesicles the polar groups remain in contact with water where, presumably, a fraction of the hydrogen bonds formed in bulk water remains.

(27) Thomas, J. K. *Chem. Rev.* **1980**, *80*, 263–299.

enthalpy and entropy changes are frequently documented in the thermodynamics of partitioning of lipophiles between water and a hydrophobic phase,[28] to our knowledge this is the first example of the compensating effects of enthalpy and entropy in the thermodynamics of an activated state involved in amphiphile partitioning.

Conclusion

This is the first systematic study of the structural determinants that control the rate of transfer of lipophiles between hydrophobic compartments separated by an aqueous space. The similarity between the effects of chain length or functional groups on the free energy of activation and the free energy of transfer from lipid to water leaves little doubt that the transfer occurs via the aqueous phase and suggests that the aqueous-phase solubility of a lipophile can be a reliable indicator of whether or not transfer can occur through water on a physiologically important time scale. Although some of the compounds we have used may be considered water insoluble by the usual criteria,[29] our study shows that many of these can still be transferred via the aqueous phase. This observation should be useful in the understanding of the mechanism by which many other compounds are transported between cellular organelles, cells, and various plasma or tissue compartments.

It is not immediately clear, on the basis of our data, why the alkyl pyrenes exhibit compensating $T\Delta S^{\ddagger}$ and $\Delta H^{\ddagger}$ changes with increasing chain length whereas the remaining pyrene-labeled lipophiles that are amphiphilic do not. We have constructed a schematic representation of the reaction coordinates for the transfer of polar and aliphatic lipophiles shown in Figure 8. It is probable that the latter remain at the lipid surface with their polar moieties exposed to the aqueous phase. In contrast, the alkyl pyrenes are probably localized in the middle of the bilayer where they might undergo quasi-crystallization, or cocrystallization with the methyl terminal ends of the phospholipid acyl chains. Presumably, this is an exothermic process with a compensating $T\Delta S$ term. The localization of hydrocarbons in the middle of a lipid bilayer is well documented[30] and it is probably that this is due to the more hydrophobic or hydrocarbon-like nature of this region. The amphiphiles, which are anchored at the surface, have some parts of their aliphatic chains in contact with a region of the bilayer into which there is some penetration of water; therefore, the surface region is not as hydrophobic as the middle of the bilayer and the hydrophobic effect is not fully expressed. This reasoning should apply equally well to measurements of ΔG_t and of $\Delta G^{\ddagger}$, since the initial states are the same in each case.

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Registry No. Py-$(CH_2)_7$-OH, 53595-24-7; Py-$(CH_2)_9$-OH, 72165-44-7; Py-$(CH_2)_{10}$-OH, 84811-88-1; Py-$(CH_2)_{12}$-OH, 84811-89-2; Py-$(CH_2)_6$-CO-O-CH_3, 84811-90-5; Py-$(CH_2)_8$-CO-O-CH_3, 84811-91-6; Py-$(CH_2)_9$-CO-O-CH_3, 84811-92-7; Py-$(CH_2)_6$-CO-OH, 84811-93-8; Py-$(CH_2)_8$-CO-OH, 72165-42-5; Py-$(CH_2)_9$-CO-OH, 64701-47-9; Py-$(CH_2)_{11}$-CO-OH, 69168-45-2; Py-$(CH_2)_3$-CH_3, 35980-18-8; Py-$(CH_2)_4$-CH_3, 80655-41-0; Py-$(CH_2)_8$-CH_3, 84811-94-9; Py-$(CH_2)_{10}$-CH_3, 84811-95-0; Py-$(CH_2)_{12}$-CH_3, 84811-96-1; Py-$(CH_2)_7$-CO-OH, 84811-97-2; Py-$(CH_2)_{10}$-CO-OH, 84811-98-3; Py-$(CH_2)_{12}$-CO-OH, 84811-99-4.

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