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Membrane Partitioning: Distinguishing Bilayer Effects from the Hydrophobic Effect†

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ABSTRACT: The free energy of transfer of nonpolar solutes from water to lipid bilayers is often dominated by a large negative enthalpy rather than the large positive entropy expected from the hydrophobic effect. This common observation has led to the concept of the "nonclassical" hydrophobic effect and the idea that the "classical" hydrophobic effect may not drive partitioning in many bilayer systems. We show through measurements of the heat capacity changes associated with the partitioning of tryptophan side-chain analogs into lipid bilayers and into bulk cyclohexane that the hydrophobic effect plays a crucial role regardless of the large negative enthalpy. The results emphasize that bulk-phase measurements are inadequate for describing bilayer partitioning. The experimental approach described should be generally useful for analyzing the bilayer interactions of a broad range of biologically important molecules.

An important goal in the design of drugs is efficient movement across membrane barriers. The development of effective strategies to accomplish this goal depends upon a clear understanding of the principles that govern the partitioning of nonpolar and amphipathic molecules into lipid bilayers. Experimental studies of the insertion and folding of membrane proteins, peptide-mediated membrane fusion, and the action of antibiotic peptides rely heavily on these principles as well. The major driving force for partitioning is widely assumed to be the hydrophobic effect as observed for partitioning into bulk nonpolar phases. However, evidence has accumulated during the past 20 years (Huang & Charlton, 1972; White, 1976, 1977; Simon et al., 1977, 1979; Seelig & Ganz, 1991; Marqusee & Dill, 1986) indicating that bilayer partitioning is much more complicated, as might have been anticipated from the fact that bilayers are anisotropic and chemically heterogeneous interfacial phases (Seelig & Seelig, 1977; Büldt et al., 1978; Wiener & White, 1992). The main complication concerns the relative contributions of enthalpy and entropy to the transfer free energy. For bulk phases at room temperature, entropy arising from the hydrophobic effect is dominant whereas for bilayers enthalpy is frequently dominant (Tanford, 1980; Huang & Charlton, 1972; Seelig & Ganz, 1991). This enthalpy-driven partitioning, referred to as the "nonclassical" hydrophobic effect (Huang & Charlton, 1972; Seelig & Ganz, 1991), appears to be a unique feature of solute–bilayer interactions. However, the precise interpretation and origin of the effect are unclear, and the suggestion has even been made that "classical" hydrophobic partitioning may not be operative in some, if not all, bilayer systems (Seelig & Ganz, 1991).

An important question thus arises that we address in this paper: To what extent is the partitioning of solutes into lipid bilayers determined by the hydrophobic effect, on the one hand, and the unique structure of bilayers, on the other? This question can be answered by recognizing that the characterization of complex hydrophobic processes only in terms of entropy and enthalpy can be misleading (Dill, 1990a; Privalov et al., 1990). The true hallmark of the hydrophobic effect is the large negative heat capacity change (ΔC_p) associated with the dehydration of nonpolar surface (Baldwin, 1986).

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There is thus widespread agreement that the proper way to quantitate the hydrophobic effect is through measurements of heat capacity changes (Gill et al., 1985; Spolar et al., 1989, 1992; Murphy et al., 1990; Dill, 1990b; Makhatadze & Privalov, 1990; Sharp et al., 1991). We have therefore analyzed bilayer partitioning using measurements of heat capacity changes associated with the partitioning of a series of tryptophan side-chain analogs (indole, 3-methylindole, and *N*-methylindole) into cyclohexane and into large unilamellar bilayer vesicles formed from POPC.[1] The results show that the hydrophobic effect is central to bilayer partitioning. In addition, the analysis allows the relative thermodynamic contributions of bilayer effects to be evaluated and provides information on the disposition of the indoles in bilayers.

THEORY

The free energy of transfer from water to bilayer is given by

$$\Delta G = \Delta H - T\Delta S \tag{1}$$

The changes in the enthalpy and entropy can be attributed to bilayer (bil) and hydrophobic (hf) effects. If there is a significant change in heat capacity, ΔC_p, of the system associated with the partitioning process, then one may write (Baldwin, 1986)

$$\Delta H = \Delta C_p(T - T_h) = \Delta H_{bil} + \Delta H_{hf} \tag{2a}$$

$$\Delta S = \Delta C_p \ln(T/T_s) = \Delta S_{bil} + \Delta S_{hf} \tag{2b}$$

where T_h and T_s are the temperatures at which ΔH and ΔS, respectively, equal 0. If the heat capacity of the bilayer lipids is not changed by solute partitioning,[2] then ΔC_p will arise solely from changes in solute–solvent interactions (the hydrophobic effect) so that ΔH_{bil} and ΔS_{bil} are independent of temperature. The contribution of the hydrophobic effect can then be distinguished from the bilayer effect by means of the measured values of heat capacity using the equations (Baldwin, 1986; Murphy et al., 1990; Dill, 1990b)

$$\Delta H_{hf} = (T - \tilde{T}_h)\Delta C_p \tag{3}$$

$$\Delta S_{hf} = \ln(T/\tilde{T}_s)\Delta C_p \tag{4}$$

where $\tilde{T}_h$ and $\tilde{T}_s$ are the values of temperature at which ΔH_{hf} and ΔS_{hf}, respectively, have bulk-phase-transfer values of 0. For *N*-methylindole transfer between water and cyclohexane (*vide infra*), we find $\tilde{T}_h$ and $\tilde{T}_s$ are 312 and 424 K, respectively. These values are in good agreement with $\tilde{T}_h$ = 295 K and $\tilde{T}_s$ = 420 K (recalculated using Flory–Huggins formalism discussed below) reported by Baldwin (1986) for alkanes, benzene, and alkylbenzenes.

Our results, described below, indicate that the indoles are located in the interfacial region of the bilayer where they have access to both the bilayer hydrocarbon region and the aqueous phase. For convenience, we will speak of "burying" the indoles there because of differences between the heat capacities associated with transfer to bulk and bilayer phases. However, the structure and composition of the interfacial region are extremely complex, and simplistic views of burying should be avoided. Structural studies of fluid bilayers show that the two bilayer interfaces, defined as the regions of the bilayer occupied by the waters of hydration of the headgroups, have a combined thickness equal to that of the hydrocarbon core (~30 Å) and contain portions of the phospholipids' methylene, glycerol, carbonyl, and phosphocholine groups (Wiener & White, 1992). Because this heterogeneity arises from thermal motion, Wiener and White (1992) describe the interfaces as regions of "tumultuous chemical heterogeneity". Operationally, burying simply means dehydration of the solute's nonpolar surface by some process that could include the headgroup region (Jacobs & White, 1989) as well as the bilayer's hydrocarbon core.

The heat capacity change, ΔC_p, for the transfer of an amphiphilic compound from water to a bulk nonpolar phase generally has a negative contribution from the nonpolar moiety and a positive contribution from the polar moiety (Makhatadze & Privalov, 1990; Spolar et al., 1992). Because our data indicate that the polar NH groups of indole and 3-methylindole remain in contact with the aqueous phase, we assume that the water-to-bilayer ΔC_p arises only from the nonpolar component of the solute. This result, used in conjunction with the water-to-cyclohexane values of ΔC_p, makes it possible to estimate the *apparent* percentage of the nonpolar surface that is buried in the bilayer. Because ΔC_p for nonpolar solutes is proportional to solute surface area (Gill et al., 1985), the fraction of the nonpolar surface buried is given by the ratio of the heat capacity for bilayer partitioning to the heat capacity for bulk-phase partitioning. We assume that the bulk-phase ΔC_p for *N*-methylindole [–79 cal/(mol·K), Table I] represents the dehydration of all of the nonpolar surface of *N*- or 3-methylindole. ΔC_p of indole is assumed to be smaller than that of *N*-methylindole by 9 cal/(mol·K) = ΔC_p(toluene) – ΔC_p(benzene) [see Privalov and Gill (1988)]. Before eqs 1–4 can be used to assess the hydrophobic and bilayer effects, two issues require attention: (1) the units used for calculating partition coefficients (Sharp et al., 1991) and (2) the interfacial properties of lipid bilayers that give rise to strong thermal expansion effects (Marqusee & Dill, 1986).

Partition Coefficient Units. Although the entropy of mixing (cratic) term is frequently accounted for by using mole fraction or volume fraction based partition coefficients (Tanford, 1980; Ben-Naim, 1978), recent experimental and theoretical work suggests that the Flory–Huggins-corrected volume fraction partition coefficients are best for comparing transfer free energies between bulk phases that have large molar volume differences (De Young & Dill, 1990; Sharp et al., 1991). The free energy of transfer from pure "liquid" (l) solute to a mixture (m) of solute (s) with solvent (a) is $\Delta G_{l\to m} = -RT \ln(\phi_s) - RT(1 - \bar{V}_s/\bar{V}_a)$, where ϕ_s is the volume fraction of solute in the mixture and the $\bar{V}$ represents partial molar volumes. The free energy of transfer of the solute from solvent w (water) to solvent b (bilayer) is thus given by

$$\Delta G_{w\to b} = -RT \ln\left(\frac{\phi_s^b}{\phi_s^w}\right) + RT\bar{V}_s\left(\frac{1}{\bar{V}_b} - \frac{1}{\bar{V}_w}\right) \tag{5}$$

where $\bar{V}_b$ is understood to be a partial molar volume appropriate

[1] Abbreviations: POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; N-MI, 1-methylindole, referred to for additional clarity as *N*-methylindole; 3-MI, 3-methylindole.

[2] The absolute heat capacity of fluid-phase bilayers is independent of temperature (Blume, 1983; Marsh, 1990), indicating that temperature-induced changes in bilayer (conformational, translational, rotational degrees of freedom, packing density, hydration, etc.) do not make a net contribution to the molar heat capacity of pure lipid bilayers. We assume that lipid structural changes induced by solutes do not change the molar heat capacity except possibly through changes in the amount of nonpolar lipid surface exposed to water. Although no data are available to prove or disprove the correctness of this assumption for bilayers, ΔC_p for the partitioning of hydrophobic solutes between polar mobile phases and hydrophobic stationary phases in liquid chromatography experiments is largely unaffected by the stationary-phase bonding (surface) density (Cole et al., 1992).

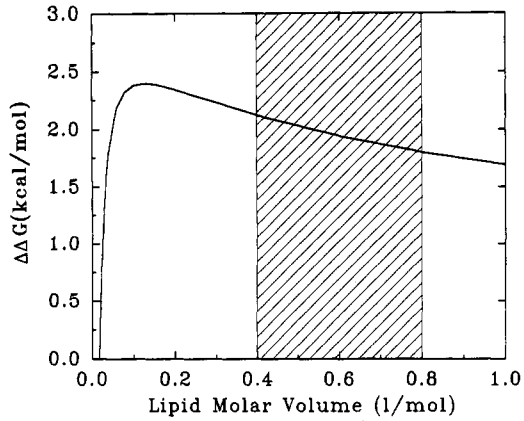

FIGURE 1: Incremental change in the free energy of transfer of *N*-methylindole as a function of the molar volume of the lipid caused by the use of Flory–Huggins-corrected partition units. $\Delta\Delta G = \Delta G_{MF} - \Delta G_{FH}$, where MF means mole fraction units and FH means Flory–Huggins units. ΔG_{MF} is independent of lipid molar volume. The value of the molar volume of *N*-methylindole used in the calculation is that of Wimley and White (1992). The volume of a POPC molecule is about 1300 Å^3, corresponding to 0.8 L/mol (Wiener & White, 1992). Approximately one-half of this volume can be attributed to the carbonyl, glycerol, and headgroup regions (Wiener & White, 1992). Amphipathic solutes such as the indoles probably sample this region as well as some part of the hydrocarbon so we have assigned an intermediate value of 0.6 L/mol for the lipid phase. This figure shows that the exact value of this parameter has little effect on the calculated free energies.

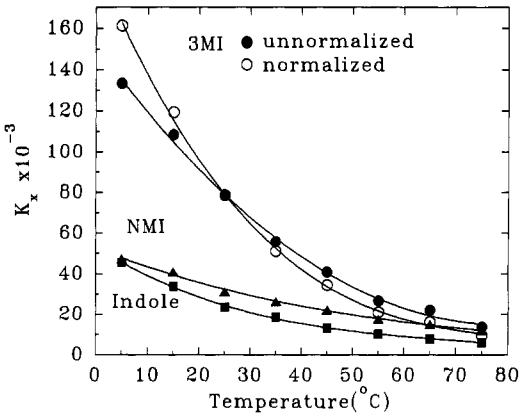

FIGURE 2: Temperature dependence of the mole fraction partition coefficients for 3-methylindole (3MI), *N*-methylindole (NMI), and indole uncorrected for lipid surface density (solid symbols). The effect of normalizing for lipid surface density in the case of 3MI is shown by the open circles. K_X is the equilibrium ratio of the mole fraction of solute in the bilayer phase to the mole fraction in the aqueous phase. Free and bound concentrations of indoles were determined by equilibrium dialysis. Surface density $\sigma = A_0/A$, where A is the area per lipid in the fluid bilayer and A_0 is the area per lipid in the crystalline state.

for the lipid molecules of the bilayer and ϕ_s^b/ϕ_s^w is the volume fraction partition coefficient. The right-most term is the correction for the molar volume differences between solute and solvents. Although the value and meaning of this formalism are still being debated (Holtzer, 1992), we note that the broad conclusions of this paper do not depend upon it; similar conclusions are reached using mole fraction or volume fraction units.

Using this approach for bilayers, however, is complicated by the fact that solutes are not uniformly distributed throughout the bilayer phase (White et al., 1981; Jacobs & White, 1989). What molar volume is appropriate for the "solvent" in such cases? Figure 1 shows that the exact value of this volume has little effect on the free energy of transfer (ΔG) for bilayer-phase molar volumes above approximately 0.2 L/mol, which corresponds to approximately 25% of the bilayer volume. On the basis of structural studies of bilayers, we estimate that apparent molar volumes of 0.4–0.8 L/mol are reasonable values for a wide range of solutes (Wiener & White, 1992). Here we use an intermediate value of 0.6 L/mol.

Interfacial Thermal Expansion Effects. Dill and De Young (1988, 1990) have shown that the temperature dependence of benzene and hexane partitioning into lipid bilayers can be entirely accounted for through bilayer surface density. We use their benzene and hexane partitioning data and lipid surface density estimated from ^{2}H-NMR order parameters to normalize the indole partitioning data to the bilayer surface density at 25 °C. This procedure allows one to account for the relative contribution of bilayer expansion to the temperature dependence of the partition coefficients. De Young and Dill (1990) define surface density $\sigma = A_0/A$, where A is the area per lipid in the fluid bilayer and A_0 is the area per lipid in the crystalline state. For phospholipids with fully saturated acyl chains, A_0 = 40 Å^2 and $A \sim 70$ Å^2 so that $\sigma \sim 0.6$. However, NMR experiments determine σ directly. The temperature dependence of the surface density of POPC was estimated by the method De Young and Dill (1988) using the ^{2}H-NMR data of Seelig and Seelig (1977) (σ = 0.61 at 25 °C). The normalized partition coefficient is given by $\tilde{K}_X(T) = K_X(T)[K_{hc}(\sigma_{298})/K_{hc}(\sigma_T)]$, where K_{hc} is the hydrocarbon (benzene or hexane) partition coefficient at the lipid surface density σ_T at temperature T (kelvins) and $K_X(T)$ is the measured partition coefficient.

MATERIALS AND METHODS

Partition coefficients were determined using equilibrium dialysis and high-pressure liquid chromatography (HPLC). Dialysis cell halves were separated by a Spectrapor 2 dialysis membrane and contained either 2–4 mM POPC or buffer (10 mM HEPES/50 mM KCl, pH 7.0) and 20–40 μM indole compound. Unilamellar vesicles of POPC (0.1-μm diameter) were prepared by extrusion (Mayer et al., 1986). Equilibrium was reached within 5 h, but experiments were run for 7 h or longer. The relative concentrations of indoles were determined by HPLC using a 5 × 0.46 cm C4 silica reverse-phase column and water/acetonitrile gradients. Aliquots containing between 1 and 10 nmol of indole compound were loaded, and absorbance was monitored at 280 nm. Peak areas were linear functions of the amount of indole compound injected. The presence of lipid had no effect on the retention time or absorbance.

The heat capacities, enthalpies, and entropies associated with partitioning into the bilayer were determined from the temperature dependence of ΔG (from eq 5) by means of nonlinear least-squares fitting to $\Delta G = \Delta C_p(T - T_h) - T\Delta C_p \ln(T/T_s)$ (see eqs 1 and 2). ΔH_{hf} and ΔS_{hf} were calculated from eqs 3 and 4 using values of $\tilde{T}_h$ and $\tilde{T}_s$ determined from the partitioning of *N*-methylindole into cyclohexane (see Theory section above).

RESULTS AND DISCUSSION

The partition coefficients for the indoles are plotted as a function of temperature in Figure 2. Shown in Table I are the calculated thermodynamic parameters for the transfer of each indole compound from water to cyclohexane (designated as bulk) and to POPC bilayers. There are two bilayer entries: bilayer (u) and bilayer (n) that refer respectively to values unnormalized and normalized for the lipid surface density effect. Importantly, all of the compounds have significant negative water-to-bilayer ΔC_p values that are not strongly

Table I: Thermodynamic Parameters for the Transfer of *N*-Methylindole (N-MI), 3-Methylindole (3-MI), and Indole from Water to Bulk Cyclohexane and to POPC Lipid Bilayers Unnormalized (u) and Normalized (n) for Lipid Surface Density at T = 298 K

solute	phase	ΔC_p [cal/(mol·K)]	% buried	ΔG^a (kcal/mol)	ΔH^b (kcal/mol)	ΔS^c [cal/(mol·K)]
N-MI	bulk[d]	−79 ± 15		−7.16	+1.11	+27.7
	bilayer (u)	−28 ± 11	35 ± 16	−8.08	−3.45	+15.5
	bilayer (n)	−26 ± 11	33 ± 15	−8.10	−5.05	+10.2
3-MI	bulk[d]	−47 ± 10		−5.95	+2.42	+28.1
	bilayer (u)	−67 ± 15	85 ± 25	−8.60	−5.42	+10.7
	bilayer (n)	−65 ± 15	82 ± 25	−8.61	−7.01	+5.4
indole	bulk[e]	−45 ± 3		−4.36	+2.56	+23.2
	bilayer (u)	−18 ± 11	25 ± 17	−7.61	−5.37	+7.5
	bilayer (n)	−16 ± 11	23 ± 14	−7.63	−6.96	+2.2

[a] Standard deviation of values 0.02–0.03 kcal/mol. [b] Standard deviation of values 0.6 kcal/mol. [c] Standard deviation of values 2 cal/(mol·K). [d] Bulk phase: Cyclohexane. Results recalculated from the full data set of Wimley and White (1992) using eqs 3 and 4. [e] Bulk phase: Cyclohexane. Data obtained using methods described by Wimley and White (1992).

affected by partition coefficient unit choice or by the surface density normalization. These results are not peculiar to the indoles; we have obtained a value of greater than −100 cal/(mol·K) for the partitioning of the hydrophobic pentapeptide *N*-acetyl-WLWLL-OH into POPC bilayers (unpublished observation), and values ranging from −32 to −130 cal/(mol·K) have been measured for the partitioning of anionic and cationic amphiphiles into highly curved, sonicated vesicles (Flewelling & Hubbell, 1986a; Seelig & Ganz, 1991). Taken together, these heat capacity changes indicate that the hydrophobic effect contributes significantly to bilayer partitioning in both planar and highly curved bilayers for a wide variety of compounds. Furthermore, the differences between the bilayer and bulk values of ΔC_p suggest that all of the indoles examined prefer the hydrocarbon–water interface of the bilayer. For *N*-methylindole, the most hydrophobic of the compounds, the bulk value of ΔC_p is −79 ± 15 cal/(mol·K) whereas the bilayer value is −28 ± 11 cal/(mol·K), indicating that only 20–50% of the nonpolar surface is removed from contact with the aqueous phase.[3] For 3-methylindole, the bilayer ΔC_p of −67 ± 15 cal/(mol·K) is larger than for the bulk-phase ΔC_p of −47 ± 10 cal/(mol·K) and approaches the bulk-phase value of *N*-methylindole. The difference in the bulk-phase values must arise from the positive contribution of the –NH group to ΔC_p (Makhatadze & Privalov, 1990). These observations thus indicate that the –NH group of 3-methylindole maintains access to the aqueous phase while 60–100% of the nonpolar surface is buried in the bilayer hydrocarbon. As might be expected from the absence of the methyl group, the ΔC_p values for indole are smaller than for the other two compounds, and indole has about 10–40% of its nonpolar surface buried. The extent of burial for the indoles strongly suggests that they are located at the hydrocarbon–water interface. This conclusion is supported by fluorescence quenching experiments.[4]

The water-to-bilayer values of ΔG, $-T\Delta S$, and ΔH unnormalized and normalized for surface density effects are compared graphically in Figure 3. The normalization is equivalent to accounting for the contribution of thermal expansion to the thermodynamic parameters. The relative contributions of the hydrophobic effect (solid bars) and bilayer effect (open bars) calculated using eqs 1–4 are illustrated.[5] Although the hydrophobic effect is not necessarily dominant, it clearly contributes significantly to the partitioning free energy.

The proposal has been made that the hydrophobic contribution to bilayer partitioning is eliminated by the rebinding of "hydrophobic effect water" to the bilayer hydrocarbon surface so that the entropy associated with bilayer partitioning arises from the disordering of acyl chains rather than from the release of hydration shell water (Seelig & Ganz, 1991; Beschiaschvili & Seelig, 1992). However, a simple geometrical argument makes this seem unlikely. The amount of additional bilayer hydrocarbon exposed upon dissolution of the solute depends upon how the bilayer responds to the solute and how much of the solute is buried. If the solute is completely buried, the bilayer could respond at one extreme by keeping the area per lipid fixed and changing the thickness d_{hc} of the hydrocarbon region. At the other extreme, d_{hc} could remain fixed and the area per lipid could increase by ΔA_{bil} for each solute added. The former case would lead to a maximum hydrophobic effect while the latter case would null the hydrophobic effect only if ΔA_{bil} coincided precisely with the surface area of the solute. Suppose the solute is spherical with surface area $A = 4\pi r^2$. For ideal volumetric mixing, the area of hydrocarbon exposed would be $\Delta A_{bil} = (4\pi r^3)/3d_{hc}$. Consequently, the amount of hydrocarbon exposed could be no larger than $\Delta A_{bil} = (r/3d_{hc})A$ for complete burying. The exposure would be even smaller if the molecule is nonspherical. But, irrespective of these arguments, the "rebinding" explanation demands that $\Delta C_p = 0$, which is inconsistent with experiment.

What is the origin of the large favorable enthalpy associated with bilayer partitioning? As can be seen from the data in Figure 3, the enthalpy cannot be explained by surface density effects that generally act to raise the entropic contribution to ΔG at the expense of enthalpy. Nor can it be explained by

[3] Changes in the exposure of lipid nonpolar surface could possibly contribute to the change in total exposed nonpolar surface. This does not affect the thermodynamic calculations since ΔC_p depends only on the net change in exposed surface. However, changes in the exposure of bilayer nonpolar surface would affect the estimate of the amount of solute nonpolar surface buried. The apparent area buried is the sum of the actual area buried and the change in lipid hydrocarbon exposure per solute. The apparent area buried is thus an approximation of the actual area buried.

[4] Fluorescence intensity of the indoles partitioning into bilayers was measured in the presence of 0–8 mol % or 5- or 16-doxylstearic acid. The quenching constant is the slope of normalized fluorescence vs mole fraction of spin label. All three indole compounds had quenching constant ratios (5-/16-position) of 1.4 ± 0.1, indicating an average position closer to the interfacial region than the hydrocarbon center. Diphenylhexatriene had a ratio (5-/16-position) of 0.6, consistent with its location deep within the hydrocarbon core of the bilayer.

[5] The relative contributions of the bilayer and hydrophobic effects will be changed if the bilayer contribution to ΔC_p is not 0. Although we do not believe the bilayer contributes significantly to ΔC_p (footnote 2), we cannot absolutely rule out the possibility. If the bilayer's contribution was between +20 and −20 cal/(mol·K), the percent burying of the solute in the bilayer (Table I) would be in error by about ±25% and $T\Delta S_{bil}$ and $T\Delta S_{hf}$ (Figure 3) would be in error by about 2 kcal/mol. ΔH_{bil} would be unaffected because ΔH_{hf} is close to 0 at the reference temperature of 25 °C. Errors of these magnitudes do not invalidate the major conclusions of this paper.

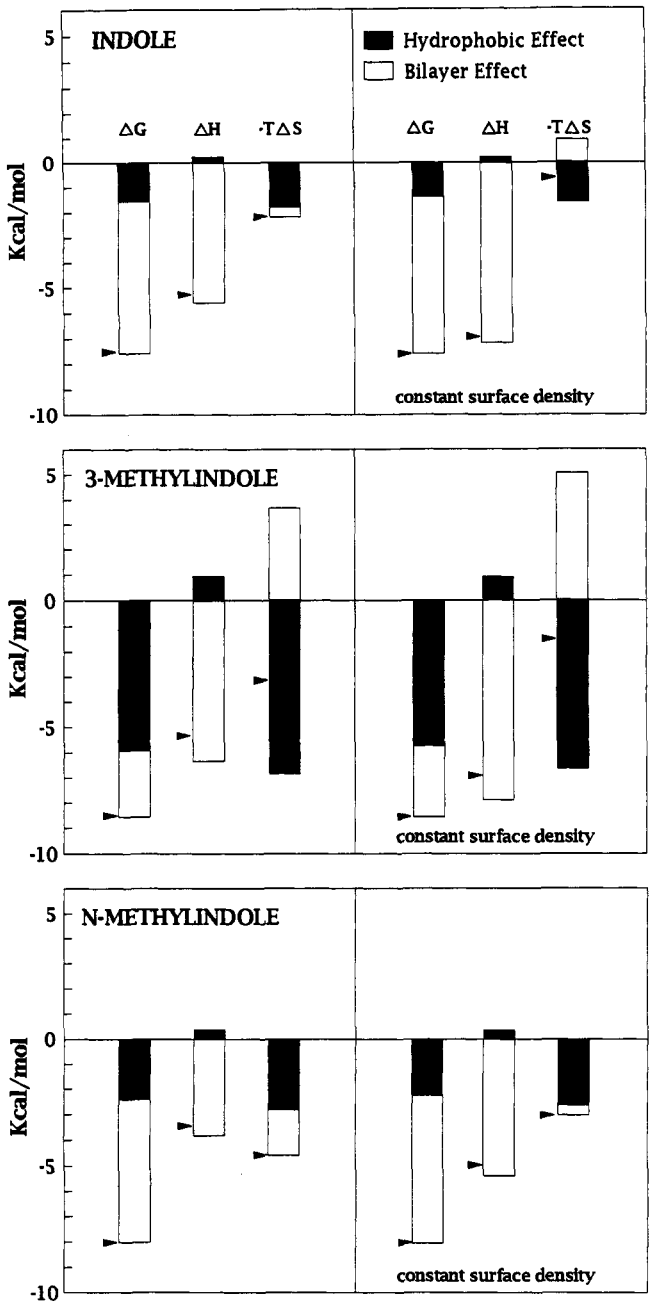

FIGURE 3: Summary of the thermodynamics of transfer of the indole compounds from water to bilayers unnormalized (left-hand data) and normalized (right-hand data) for surface density effects. The calculated contributions of the hydrophobic effect (black bars) and bilayer effect (white bars) to the free energy, enthalpy, and entropy are shown. The arrows indicate the net values. The large enthalpies are due to bilayer effects which may make positive or negative contributions to the entropy. The normalized data show what thermodynamic values would be expected if partitioning occurred at fixed lipid surface density, *i.e.*, in the absence of normal thermal expansion. Thus, a comparison of the normalized with the unnormalized data reveals the contribution of thermal expansion to the thermodynamics of partitioning.

the hydrophobic effect because Figure 3 shows that hydrophobic effect contributions to enthalpy are small and unfavorable. Seelig and Beschiaschvili (1992) have suggested that the large enthalpies are due to van der Waals interactions resulting from solute-induced changes in lipid acyl chain packing and have discussed the enthalpy "potential" of lipid bilayers from a consideration of the enthalpy of alkane solid-to-liquid and bilayer gel-to-liquid-crystal phase transitions that is estimated to be 0.5 kcal/mol of $-CH_2-$. Our observed surface density-corrected enthalpies of −5 to −7 kcal/mol of indole compound would correspond to solidifying 10–14 mol of CH_2/mol of indole. Although reversed-phase liquid chromatography experiments support this proposal qualitatively (Cole et al., 1992), the evidence for it is largely circumstantial.

Another possibility is dipolar interactions between the lipid and solute dipole moments. The net dipole moment (normal to the bilayer) of a lipid molecule is 1–2 D (positive inside; 1 D = 3.336×10^{-30} C·m) and arises mainly from the vector sum of the dipolar contributions from the water and carbonyl groups (Flewelling & Hubbell, 1986b) that each have total dipole moments of about 2 D. The dipole moment of each of the indoles is also about 2 D (McClellan, 1963). Interactions between solute dipoles and the electrostatic field produced by the lipid dipoles (maximum 10^8 V/m = 10 mV/Å) can only provide interaction energies $W = \vec{\mu}\cdot\vec{E}$ of no more than −0.5 kcal/mol. The energy of specific interactions between dipoles was calculated either by the classical expression $W = \mu_1\mu_2/\epsilon\epsilon_0 r^3$ or, more realistically, by modeling the dipoles as pairs of partial charges (0.05–0.2 e) separated by 1–5 Å such that the dipole moment (the product) is 1–2 D. The dielectric constant was assumed to be in the range 2–10. Both methods indicate that potential energies of more than −5 kcal/mol are possible, but only if the dipoles are within 2–5 Å and are, on average, roughly antiparallel.

Jacobs and White (1989) showed that tryptophan-containing tripeptides localize into the interfacial bilayer region but assumed that this occurred because the peptide backbone's polar groups opposed dissolution. The present results show, however, that the isolated tryptophan side chain prefers the interfacial region despite the fact that the cost of burying the –NH of tryptophan in a bulk nonpolar phase is small compared to the free energy loss that can be provided by the hydrophobic effect (Wimley & White, 1992). The fact that *N*-methylindole also prefers the interface strongly suggests that dipole interactions may be important for interfacial stabilization. Interestingly, because both the photosynthetic reaction center of *Rhodobacter sphaeroides* (Allen et al., 1987) and the bacterial outer-membrane pore-protein porin (Weiss et al., 1991) are enriched in tryptophan and tyrosine residues near the headgroup region of the lipid bilayer, Schiffer et al. (1992) have proposed that tryptophan and tyrosine residues play a critical role in the translocation of transmembrane protein segments.

The net thermodynamic parameters for the partitioning of our indole compounds into bilayers have the same general features observed for a wide range of other amphiphilic compounds. We therefore believe that the experimental approach and results presented in this paper will be generally applicable to a wide range of compounds of biological interest. The general scheme for the partitioning process that emerges from the analysis of our data is shown in a highly schematic fashion in Figure 4. We suggest that exclusion of nonpolar solutes from water is a fundamental driving force for partitioning but that specific solute–bilayer interactions and the response of the bilayer to partitioning modify the overall thermodynamics of the process. The results raise important questions about the nature of the partitioning of proteins into bilayers. Most thermodynamic analyses of protein folding in membranes treat the bilayer interior as equivalent to a bulk hydrocarbon phase and assume that the hydrophobic effect is the sole driving force for partitioning (Engelman & Steitz, 1981; Jähnig, 1983; Jacobs & White, 1989). It is now clear, however, that one must take into account the "bilayer effect". The only way to do this at the moment is by means of

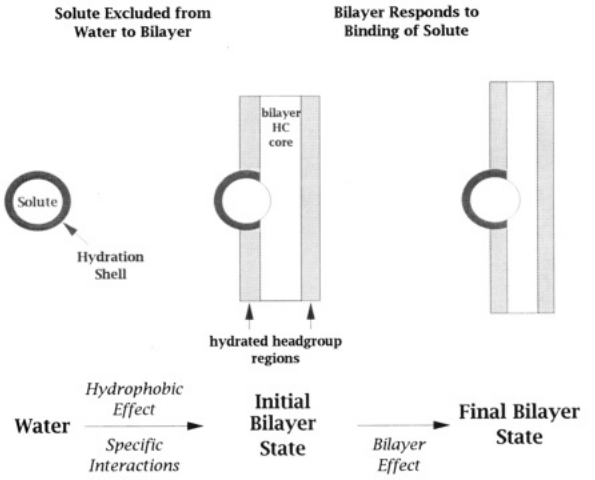

FIGURE 4: A highly schematic representation of the partitioning of solutes from water into bilayers. The significant heat capacities associated with partitioning indicate that the hydrophobic effect is operative in lipid bilayer partitioning. We suggest that the solute is initially excluded from the aqueous phase to the bilayer by the hydrophobic effect. The partitioning of the solute into the bilayer leads to changes in the bilayer organization that contribute bilayer effects to the thermodynamics of partitioning. The change in thickness of the bilayer should not be taken literally; it is used only to signify unknown changes in bilayer state.

experiments, but one can hope that useful general rules will emerge from systematic measurements.

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