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Nonclassical Hydrophobic Effect in Membrane Binding Equilibria†

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ABSTRACT: The enthalpy of transfer of four different amphiphilic molecules from the aqueous phase to the lipid membrane was determined by titration calorimetry. The four molecules investigated were the potential-sensitive dye 2-(*p*-toluidinyl)naphthalene-6-sulfonate (TNS), the membrane conductivity inducing anion tetraphenylborate (TPB), the Ca^{2+} channel blocker amlodipine [Bäuerle, H. D., & Seelig, J. (1991) *Biochemistry 30*, 7203-7211], and the positively charged local anesthetic dibucaine. All four amphiphiles penetrate into the hydrophobic part of the membrane, and their binding constants, after correcting for electrostatic effects, range between 600 M^{-1} for dibucaine and 60 000 M^{-1} for tetraphenylborate. The corresponding changes in free energy were about −6 to −9 kcal/mol. Binding of the amphiphiles to membrane vesicles composed of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine was accompanied by exothermic heats of reaction for all four molecules. For TNS, TPB, and amlodipine, the enthalpies of transfer were almost identical and corresponded to $\Delta H \approx -9$ kcal/mol, essentially accounting for the total free energy change. Thus, the binding of these charged amphiphiles to the hydrophobic membrane was driven by enthalpy. This is in contrast to the classical hydrophobic effect, where the transfer is considered to be entropy driven. For dibucaine, the enthalpy of transfer was smaller with $\Delta H \approx -2$ kcal/mol but was still about one-third of the total free energy change. All enthalpies of transfer exhibited a distinct temperature dependence with molar heat capacities ΔC_p of −30 to −100 cal mol^{-1} K^{-1} for the transfer from water to the membrane. These molar heat capacities are quite large and correspond to those obtained for the partitioning of small organic molecules between water and a pure organic phase. The nonclassical hydrophobic binding of the amphiphiles is explained by van der Waals interactions between the nonpolar residues of the solute and the hydrophobic core of the lipid bilayer.

The interaction of nonpolar molecules such as hydrophobic peptides or drugs with the lipid membrane is usually ascribed to the *hydrophobic effect*. The hydrophobic residues of these molecules tend to avoid contact with the aqueous environment and penetrate into the hydrophobic core of the membrane. It is also common thinking to consider the hydrophobic effect as an *entropic* phenomenon, i.e., the driving force for the association is the release of water molecules from the nonpolar surface of the solute [cf. Tanford (1980)]. The entropic interpretation of the hydrophobic effect is based on measurements of the water solubility of small organic molecules such as benzene or hexane as a function of temperature. It was found that the entropy of transfer, ΔS, of these compounds from the pure liquid state to water was large and negative (at room temperature), whereas the corresponding enthalpy, ΔH, was approximately zero or only slightly negative. The low solubility of nonpolar molecules in the aqueous phase is thus caused by a negative excess entropy. This experimental finding has led to a specific molecular picture for the hydrophobic effect: the insertion of the nonpolar molecule in water is assumed to produce an ordering of the water molecules around the solute such that the perturbation of the hydrogen-bonding pattern of water is minimized. The nonpolar molecule and its hydration sphere constitute an "iceberg" swimming in the aqueous phase. The ordering of the water molecules by the nonpolar solvent explains the large negative entropy of transfer. The same picture also explains the second characteristic feature of the hydrophobic effect, namely, its strong dependence on temperature. For nonpolar substances, the heat capacity, ΔC_p,

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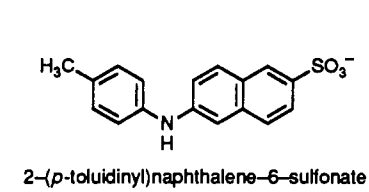
of transfer from the pure liquid to water is large and positive, much larger than that of the pure nonpolar liquid itself. The additional heat is thought to be consumed by the "melting" of the iceberg with increasing temperature.

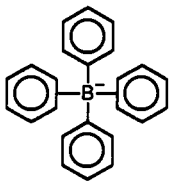
The conventional interpretation of the hydrophobic effect ignores the van der Waals attraction energy [cf. Privalov and Gill (1989)]. The very existence of a liquid phase of benzene or hexane at room temperature demonstrates the presence of strong van der Waals attractions between these molecules. An approximate measure of this interaction energy is the heat of evaporation, which for benzene, for example, is $\Delta H_{vap} \approx +6$ kcal/mol. In order to allow for the van der Waals energy in the transfer of a nonpolar molecule to water, we may consider the following two-step process. First, the nonpolar liquid is dissociated into individual molecules at the expense of the van der Waals energy (positive ΔH). Second, the nonpolar molecules are condensed into the aqueous phase. Since the overall enthalpy is close to zero (at room temperature), the hydration step must be associated with a negative hydration enthalpy (Privalov & Gill, 1989). Indeed, the heat of hydration of apolar substances such as methane or ethane has been measured and was found to be large and negative [cf. Cantor and Schimmel (1980)]. Enthalpic contributions to the hydrophobic effect are further suggested by the observation that at high temperatures the aversion of nonpolar solutes for water is driven exclusively by enthalpy (at 140 °C) whereas the entropy of transfer equals zero (Baldwin, 1986; Privalov & Gill, 1989; Dill, 1990).

The partitioning (adsorption/binding) of amphiphilic molecules such as potential-sensitive dyes, hydrophobic ions, local anesthetics, or small peptides into model membranes or biological membranes is usually also considered to be driven by entropy. However, a search of the literature reveals conflicting results on the distribution of the free energy of transfer, ΔG, between enthalpic and entropic forces. For a series of hydrophobic tripeptides, it was demonstrated that the binding to the lipid membrane is clearly an entropy effect and that the enthalpy of transfer is zero (Jacobs & White, 1989). In contrast, in binding studies of the fluorescent dye 2-(*p*-toluidinyl)naphthalene-6-sulfonate (TNS)[1] with phosphatidylcholine vesicles, a large exothermic binding enthalpy was observed whereas the entropy term was close to zero (Huang & Charlton, 1972). This led to the suggestion that the association of TNS with the membrane was of the "nonclassical" hydrophobic type interaction. Likewise, the binding of several peptides to lipids was studied by titration calorimetry. Reaction enthalpies were reported for melittin/1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) (Blume, 1988), apolipoprotein A-II/DMPC (Massey et al., 1981), apolipoprotein A-I/1,2-dimyristoyl-*sn*-glycero-3-phosphatidylglycerol (DMPG) (Epand et al., 1990), glucagon/DMPC (Epand & Sturtevant, 1981), and transcarbamylase leader peptide/phospholipid membranes (Myers et al., 1987). Most of these studies measured exothermic (negative) ΔH values, but the molecular interpretation of these results is not straightforward since either the corresponding free energies have not been determined or the binding equilibrium is masked by an exothermic phase transition of the membrane lipids.

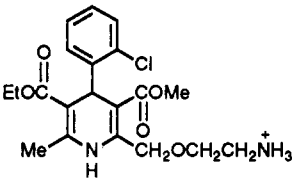
Utilizing high-sensitivity titration calorimetry, we have therefore measured the binding enthalpies of several simple amphiphilic molecules interacting strongly with *fluid* lipid bilayers. Both anionic and cationic amphiphiles were chosen in order to assess the influence of charge on the enthalpy of transfer, ΔH. The following compounds were investigated:



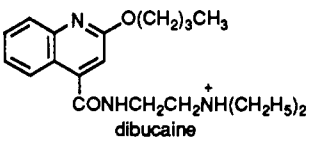
2-(*p*-toluidinyl)naphthalene-6-sulfonate



tetraphenylborate



amlodipine



dibucaine

TNS(I) was chosen in order to compare the results obtainable from the microcalorimetric titration method with previous measurements of ΔH via the temperature dependence of the binding constant (Huang & Charlton, 1972). As a second hydrophobic anion we selected tetraphenylborate (TPB) since the membrane properties of this molecule are well known from conductivity measurements (Ketterer et al., 1971). The local anesthetic dibucaine, which is positively charged at pH 5.5, served as an example for cationic amphiphiles. The binding properties of this molecule have been characterized extensively by magnetic resonance methods (Seelig et al., 1987). Finally, we have included ΔH measurements of the positively charged Ca^{2+} blocker amlodipine (Bäuerle & Seelig, 1991). All binding studies were made with small unilamellar vesicles (SUV) composed of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC).

Materials and Methods

Titration Calorimetry. Heats of reaction were measured with a Microcal MC-2 high-sensitivity titration calorimeter (Microcal, Northampton, MA) as described by Wiseman et al. (1989). The sample cell had a volume of 1.27 mL and contained unilamellar vesicles at a lipid concentration of ~10 mM in buffer (the total amount of lipid in sample cell was ~13 μmol). The lipophilic cations or anions were placed in a 100-μL syringe at concentrations of 0.5–4 mM and 5- or 10-μL injections were made every 6 min with continuous stirring. After 10 such injections, the total amount of added amphiphile was typically 0.1 μmol, i.e., the lipid concentration remained by far in excess during the whole titration experiment.

Liposome Preparation. Unilamellar vesicles were prepared by two different methods. Synthetic 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (30 mg) (Avanti Polar Lipids, Birmingham, AL) was dissolved in chloroform/methanol, and the solvent was evaporated under a stream of nitrogen. The flask was then dried for several hours in vacuo. Buffer (3 mL) (TPB was measured in 0.1 M NaCl and 10 mM Tris-HCl, pH 7.4; TNS, 0.1 M KCl and 10 mM Tris, pH 8.0; dibucaine, 0.1 M NaCl and 50 mM potassium phosphate, pH 5.5; amlodipine, 0.1 M NaCl and 10 mM Tris, pH 7.2) was added, and the dispersion was sonified for about 20 min. The opalescent solution was centrifuged to remove metal debris.

As a control, lipid vesicles of larger diameter were prepared by extrusion. A suspension of 10 mg of lipid/mL of buffer

[1] Abbreviations: TNS, 2-(*p*-toluidinyl)naphthalene-6-sulfonate; TPB, tetraphenylborate; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DMPG, 1,2-dimyristoyl-*sn*-glycero-3-phosphoglycerol; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; SUV, small unilamellar vesicles.

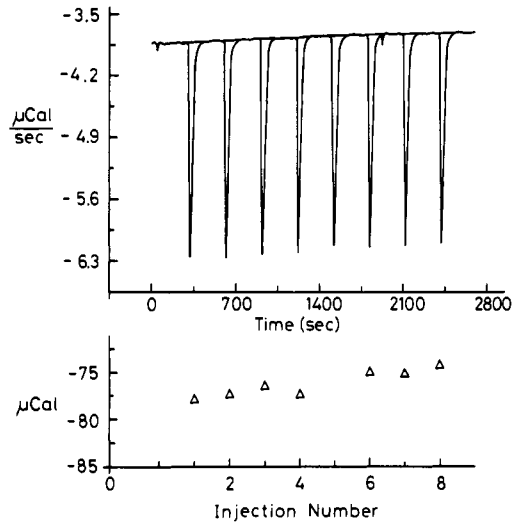


FIGURE 1: Titration calorimetry of small unilamellar vesicles of POPC (~12 mM lipid concentration) with a 0.79 mM TNS solution (27 °C; 10 mM Tris, 100 mM KCl, pH 8.0). 10 μL of TNS in buffer was injected into the calorimeter cell (1.27 mL) containing POPC vesicles in buffer. The upper part of the figure shows the calorimeter tracings; the bottom part yields the heat of reaction as evaluated from the areas under the calorimeter tracings.

was first vortexed and then subjected to several freeze–thaw cycles. The suspension was then extruded 10 times through a polycarbonate filter of 100-nm pore size. For TNS and TPB, the differences in the measured ΔH values between extruded and sonicated vesicles were of the order of ±10%.

Results

Binding of Negatively Charged TNS and TPB to POPC Vesicles. The fluorescent probe TNS adsorbs strongly on bilayer membranes and responds to electric potentials applied across the bilayer membrane. The binding equilibrium has been studied in detail by gel filtration (Huang & Charlton, 1972) and microelectrophoresis (McLaughlin & Harary, 1976). Figure 1 shows the titration of unilamellar POPC vesicles (~12 mM lipid) in buffer (100 mM KCl, 10 mM Tris, pH 8.0) with a 0.79 mM solution of TNS (concentration determined by UV spectroscopy) in the same buffer. In each step, 10 μL of the TNS solution were injected into the calorimeter cell. Since the lipid was much in excess over the added drug, each titration step produced the same reaction enthalpy ΔH, which was evaluated from the area under the calorimeter trace (cf. lower panel of Figure 1). Figure 1 yields an exothermic reaction enthalpy for the TNS transfer from water to membrane of $\Delta H = -76.14 \pm 1.4$ μcal per injection (at 27 °C). As a control, the same TNS solution was injected into buffer without lipid; the heat of dilution was found to be negligible. As a final control, a solution without TNS was injected into buffer with lipid, and the heat of dilution was also found to be negligible.

The binding constant for TNS binding to egg yolk lecithin membranes is $K \simeq 5000\ M^{-1}$ (Huang & Charlton, 1972; McLaughlin & Harary, 1976), and it can thus be expected that almost all TNS is immediately bound to the lipid vesicles, provided the equilibrium is established rapidly enough. Indeed, the calorimeter traces of Figure 1 show a constant baseline with no evidence of slow secondary reactions. With the assumption of complete TNS binding, the molar binding enthalpy is calculated to be $\Delta H = -9.0 \pm 0.7$ kcal/mol at 27 °C (average of four titration series at different concentrations of TNS). This result is in good agreement with $\Delta H = -9.5$ kcal/mol reported by Huang and Charlton (1972).

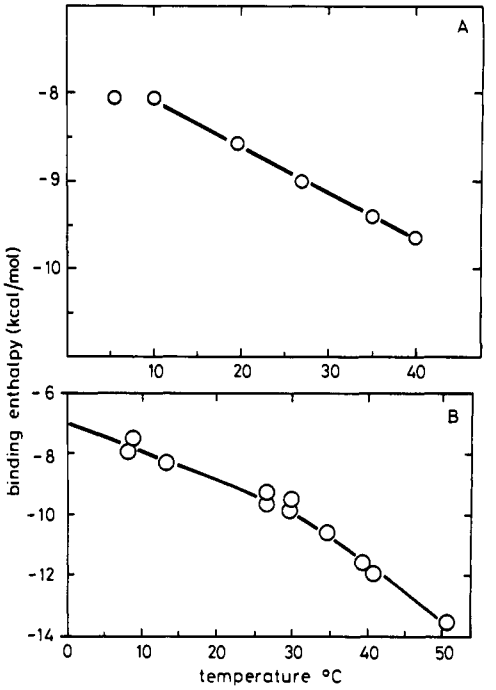


FIGURE 2: (A) Variation with temperature of the enthalpy of transfer/binding of TNS from the aqueous phase to POPC membranes. The concentration of the injected TNS solutions varied between 0.2 and 0.79 mM; the total concentration in the calorimeter cell varied between 2 and 60 μM (10 mM Tris, 100 mM KCl, pH 8.0). (B) Enthalpy of transfer/binding of negatively charged tetraphenylborate (TPB) to POPC vesicles as a function temperature. The lipid concentration was about 12 mM; the TPB concentration in the calorimeter cell was less than 70 μM at the end of the titration experiment (0.1 M NaCl, 10 mM Tris, pH 7.4).

It should be noted that the gel filtration studies of Huang and Charlton (1972) were performed with TNS concentrations in the range of 5–60 μM whereas the solutions in the calorimeter injection syringe had a concentration of 300–790 μM. However, since only 10 μL was injected into a calorimeter cell of volume $V = 1.27$ mL, the final TNS concentrations in the calorimeter cell were 2.4–5.51 μM after the first injection and 20–60 μM after 10 injections. Hence the concentration range of the two types of measurements was rather similar. It could be argued that TNS is aggregated at higher concentrations. However, as mentioned above, injection of the same TNS solutions into buffer without lipid did not result in a measurable dilution enthalpy.

We have further investigated the temperature dependence of the TNS transfer enthalpy, and the data are summarized in Figure 2A. The enthalpy of transfer is not constant but decreases smoothly with increasing temperature. Linear regression analysis of the data in Figure 2 yields a molar heat capacity of $\Delta C_p = -54$ cal mol^{-1} K^{-1} for the transfer of TNS from the aqueous phase to the bilayer.

Analogous experiments were performed with tetraphenylborate (TPB), a lipophilic anion used extensively to study membrane transport processes (Ketterer et al., 1971; Pickar & Benz, 1978; Benz, 1988). The binding constant for TPB with neutral lipid bilayers is of the order of 10^5–10^6 M^{-1} [cf. Benz (1988) and Smejtik and Wang (1990)], and charge pulse experiments reveal a rapid binding of TPB to lipid membranes (Benz, 1988). Titration of POPC vesicles (0.1 M NaCl, 10 mM Tris, pH 7.4) with tetraphenylborate solutions in the concentration range of 0.5–1 mM TPB yielded similar calorimeter tracings as shown above for TNS. At 25 °C, the enthalpy of the TPB transfer from buffer to the membrane phase was $\Delta H = -9.3 \pm 0.5$ kcal/mol. This is larger than the

Table I: Thermodynamic Parameters for the Transfer of Amphiphilic and Hydrophobic Molecules from Water to the Lipid Membrane or to Organic Liquid (at 25 °C)

solute	membrane	pH	buffer	K^a (10^3 M^{-1})	ΔG^b (kcal/mol)	ΔH (kcal/mol)	$T\Delta S$ (kcal/mol)	ΔC_p (cal/mol·K)	ref
TNS^-	POPC	8.0	0.1 M KCl/10 mM Tris	5[c]	-7.4	-9.0	1.6	-54	this work
TPB^-	POPC	7.4	0.1 M NaCl/10 mM Tris	59[d]	-8.9	-9.3	0.4	-90	this work
dibucaine$^+$	POPC	5.5	0.1 M NaCl/50 mM KPO_4	0.66[e]	-6.2	-1.9[f]	-4.3	-41	this work
amlodipine$^+$	POPC	7.25	0.1 M NaCl/10 mM Tris	15.5	-8.1	-9.2	1.1	-32	Bäuerle and Seelig (1991)
hexane	DOPC	~7	0.1 M NaCl		-6.2	-1.7	-4.5		Simon et al. (1977, 1979)
hexane	hexane		pure water		-7.7	0	-7.7	-105	Privalov and Gill (1989)
benzene	benzene		pure water		-4.6	-0.5	-4.1	-54	Privalov and Gill (1989)

[a] K refers to a partition equilibrium $X_b = KC_M$ where X_b is the mole fraction and C_M is the interfacial concentration of the solute as calculated from the Gouy-Chapman theory. [b] ΔG was calculated according to $\Delta G = -RT \ln (55.5K)$. The factor 55.5 accounts for the "cratic" contribution to the binding equilibrium [cf. Cantor and Schimmel (1981)]. [c] Binding constant taken from McLaughlin and Harary (1976). [d] Binding constant taken from Smejtek and Wang (1990); DPPC vesicles at 42 °C. [e] Binding constant taken from Seelig et al. (1987). [f] The measured enthalpy of transfer was ΔH_{min} = 1.5 kcal/mol. Because of the low binding constant, only 80% of the injected dibucaine is bound. The table lists the corrected ΔH.

enthalpy of transfer of $\Delta H \approx -2$ kcal/mol derived from charge pulse experiments with black membranes composed of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine at very low concentrations of TPB (Benz, 1988). The variation of the enthalpy of transfer with temperature is shown in Figure 2B. An even more pronounced temperature dependence is exhibited for TPB than for TNS. At low temperatures (5–30 °C), the molar heat capacity is $\Delta C_p \approx -90$ cal mol^{-1} K^{-1}; above 30 °C, this value almost doubles.

Injection of TPB solutions into buffer produces a small endothermic heat of dilution of about 0.2 kcal/mol. This correction is included in the ΔH value given above. The total TPB concentration in the calorimeter cell varied between 8 and 80 μM TPB. With phosphorus and deuterium magnetic resonance methods it could be demonstrated that the POPC membranes remained in the bilayer phase in this concentration range, in spite of the considerable amount of TPB binding (Altenbach, 1984; Malthaner, 1989).

Binding of Positively Charged Dibucaine and Amlodipine to POPC Membranes. The positively charged local anesthetic dibucaine binds to planar POPC membranes with a binding constant of K = 660 M^{-1} (at pH 5.5) (Seelig et al., 1987). The binding equilibrium appears to be established rapidly, and titrating POPC vesicles with dibucaine solutions (0.1–4 mM dibucaine, 0.1 M NaCl, 0.05 potassium phosphate, pH 5.5) leads to similar calorimeter tracings as shown for TNS. With the assumption of complete dibucaine binding (cf. below), we can calculate a minimum enthalpy of transfer $\Delta H_{min} \approx -1.5$ kcal/mol (at 25 °C and corrected for a small dilution effect), which is distinctly smaller than the ΔH values obtained for TNS and TPB. The temperature dependence of ΔH_{min} is displayed in Figure 3. The enthalpy of transfer varies linearly over the whole temperature range investigated according to

$$\Delta H_{min} \text{ (kcal/mol)} = -0.48 - 0.041(T - 273) \quad (1)$$

The minimum molar heat capacity is thus given by $\Delta C_{p,min}$ = −41 cal mol^{-1} K^{-1}.

Finally, Figure 3 also contains data measured previously for the binding of a Ca^{2+} channel blocker amlodipine to POPC membranes (Bäuerle & Seelig, 1991). The binding is characterized by a binding constant of $K \approx 16\,000$ M^{-1}, and the enthalpy of transfer is $\Delta H \simeq -9.2$ kcal/mol at 25 °C. The temperature dependence of the enthalpy of transfer is linear in the temperature range 8–40 °C according to

$$\Delta H \text{ (kcal/mol)} = -8.4 - 0.032(T - 273) \quad (2)$$

The molar heat capacity for the transfer of amlodipine from water to the membrane is thus ΔC_p = −32 cal mol^{-1} K^{-1}.

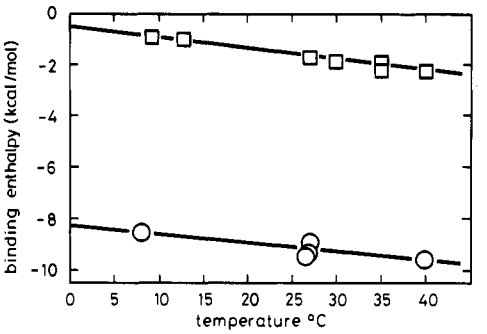


FIGURE 3: Enthalpy of transfer/binding of positively charged dibucaine (□) and amlodipine (○) to POPC vesicles. Dibucaine was measured at pH 5.5 (100 mM NaCl, 50 mM potassium phosphate), amlodipine at pH 7.2 (0.1 M NaCl, 10 mM Tris). The data for amlodipine were taken from Bäuerle and Seelig (1991).

Discussion

The thermodynamic parameters for the transfer of the four charged amphiphiles from water to the lipid bilayer are summarized in Table I. At low concentrations, the binding equilibrium of all four molecules can be described by a partition equilibrium of the form

$$X_b = KC_M \quad (3)$$

where X_b denotes the mole fraction of membrane bound amphiphile and C_M is the concentration of amphiphile free in solution, immediately above the plane of binding. For charged solutes, C_M is generally different from the equilibrium concentration and can be calculated if the membrane surface potential is known.[2] Detailed discussions based on the Gouy–Chapman theory have been published for TNS (McLaughlin & Harary, 1976), dibucaine (Seelig et al., 1987), TPB (Smejtek & Wang, 1990), and amlodipine (Bäuerle & Seelig, 1991). When C_M is employed instead of C_{eq} in the binding equilibrium, electrostatic effects are eliminated and the binding constant K can be considered as a true measure of the hydrophobic binding energy.

Inspection of Table I reveals that for all four amphiphiles studied the enthalpy change ΔH for the transfer from water

[2] The interfacial concentration C_M is related to the equilibrium concentration C_{eq} according to $C_M = C_{eq} \exp(-F_o\psi_o/RT)$, where ψ_o is the membrane surface potential, F_o is the Faraday constant, and RT has its usual meaning. ψ_o can be calculated by using the Gouy–Chapman theory.

to the membrane is distinctly negative. In fact, for three of these amphiphiles, ΔH is almost identical with or even larger than the total free energy change, ΔG. This means that the transfer of these molecules from water to the membrane is "enthalpy driven" whereas the entropy of transfer is close to zero or positive. Hence, in addition to TNS (Huang & Charlton, 1972), TPB and amlodipine constitute two further examples of the "nonclassical" hydrophobic type interaction. The comparison of the latter two molecules further demonstrates that the sign of the electric charge is of secondary importance, even though the intrinsic membrane dipole potential strongly favors the binding of the anion over the cation (Flewelling & Hubbell, 1986).

The binding constant of the dibucaine cation is distinctly smaller than those of the other hydrophobic ions, and the assumption of a complete binding of dibucaine to the lipid is no longer warranted. A quantitative analysis, combining the Henry–Dalton partition equilibrium with the Gouy–Chapman theory, demonstrates that under the specific experimental conditions employed only 80% of the total dibucaine is membrane bound. The transfer enthalpy is thus $\Delta H \simeq \Delta H_{min}/0.80$. Table I includes the corrected ΔH values, and ΔH corresponds to one-third of the total free energy change.

The thermodynamic parameters obtained for dibucaine are paralleled to some extent by those measured for truly nonpolar substances such as benzene or hexane (Simon et al., 1977, 1979). For membranes composed of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine, the free energy of transfer of hexane was found to be $\Delta G = -6.2$ kcal/mol (from water to the membrane), and the enthalpy was $\Delta H = -1.7$ kcal/mol at 25 °C. In contrast, the enthalpy of transfer from water to the pure liquid phase was zero [cf. Simon et al. (1979); Table I]. Hence, even for a completely nonpolar molecule, the transfer from water into the membrane is characterized by a more negative ΔH than the transfer from water into an organic liquid.

Spectroscopic and conductivity measurements have provided evidence that the four amphiphiles discussed above indeed penetrate into the membrane and are not just superficially adsorbed on the membrane polar region. One therefore expects that water molecules are released from the hydrophobic part of the amphiphiles, leading to a positive transfer entropy. Nevertheless, for three of these compounds $\Delta S_{transfer}$ remains close to zero. At the same time, the loss of hydration enthalpy is more than compensated by the gain in ΔH upon entering the membrane.

In order to account for these findings, the following model is suggested. The intercalation of the amphiphiles between the lipids expands the membrane and also increases the cross-sectional areas of the hydrocarbon chains by inducing a more disordered chain motion. The expansion of the membrane surface requires an additional surface hydration. As $\Delta S_{transfer}$ is close to zero, this suggests that the entropy *increase* due to a release of water molecules from the solute is compensated by an equally large entropy *loss* due to a binding of water molecules to the membrane surface. By the same mechanism most of the hydration energy of the amphiphile could be recovered. The experimentally observed large negative ΔH would then essentially reflect the van der Waals interaction between the nonpolar part of the amphiphiles and the membrane hydrocarbon core.

A further point of interest is the temperature dependence of ΔH. For *simple* solutions the transfer from one phase to the other is characterized by an enthalpy and an entropy change, both of which are temperature independent (Dill, 1990). Consequently, the heat capacity of transfer, ΔC_p, is small. For hydrophobic solutes, however, ΔH varies considerably with temperature, and ΔC_p is large. Since the molecular model of the hydrophobic effect is based on an ordering of the water molecules around the nonpolar solute, the large heat capacity ΔC_p is usually explained by a gradual melting of the "iceberg" structure, leading to a high-energy, high-entropy state of the released water molecules. As far as ΔC_p is concerned, the membrane partition equilibria investigated here exhibit a similar tendency. All four amphiphiles are characterized by a distinct temperature dependence of their enthalpies of transfer. With increasing temperature ΔH becomes more negative, i.e., the transfer from water to membrane is energetically more favorable. The molar heat capacities are large (last column of Table I) and are in the range typically quoted for hydrophobic equilibria (Privalov & Gill, 1989).

"Classic" hydrophobic equilibria and "nonclassic" membrane partition equilibria differ, however, with respect to the temperature dependence of ΔC_p. In the conventional benzene–water partition equilibrium, the heat capacity change of the transfer reaction decreases with increasing temperature, usually explained by a decreasing number of water molecules constituting the "iceberg" [cf. Privalov and Gill (1989), Figure 2]. In the present studies, ΔC_p remains fairly constant in the temperature interval investigated.

Conclusions. The penetration of amphiphilic drugs into lipid bilayers is associated with a distinctly negative enthalpy of binding. In fact, for three of the four amphiphiles studied, the measured ΔH accounts for the total free energy change. The binding enthalpy appears to be independent of charge since anionic (TPB and TNS) and cationic (amlodipine) molecules yield similar ΔH values. On the other hand, the observation of large negative ΔH values is not limited to simple organic amphiphiles. Titration calorimetry of two amphiphilic peptides, namely a cyclic analogue of somatostatine (G. Beschiaschvili and J. Seelig, unpublished) and a substance P antagonist (R. Lehrmann, A. Seelig, and J. Seelig, unpublished) with POPC vesicles revealed binding enthalpies of $\Delta H \simeq -6$ kcal. Since the binding constants of the cyclic somatostatin analogue is $K \approx 100\ M^{-1}$ (Beschiaschvili & Seelig, 1990) and that of the substance P antagonist is $K \approx 1000\ M^{-1}$ (A. Seelig, in preparation), the enthalpy is again the driving force for the transfer of the peptides from the aqueous phase to the membrane.

In conclusion, the present findings suggest that the entropy-driven binding of amphiphilic substances to membranes may be less common than generally anticipated. In most of the samples studied so far with titration calorimetry, the enthalpy of transfer appears to be the dominant energy factor. A molecular interpretation of the thermodynamic data is difficult, but it is tempting to suggest that the observed ΔH values mainly reflect the van der Waals interaction energy between the hydrophobic residues of the solute and the hydrophobic core of the membrane.

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Lateral Proton Conduction in Mixed Monolayers of Phosphatidylethanolamine and Cetyltrimethylammonium Bromide

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ABSTRACT: Proton conduction is known to be facilitated along phospholipid monolayers spread on aqueous phases. This property was monitored with mixed cetyltrimethylammonium bromide/phosphatidylethanolamine monolayers. The film was shown to be metastable by surface pressure and fluorescence measurements. The detergent was leaving the interface for the bulk phase. Nevertheless, a fraction of the detergent remained in the lipid matrix, as shown by the binding of the fluorescent probe 8-anilino-1-naphthalenesulfonate. Its dissociation constant decreased, and the nature of its binding site was affected, as shown by a shift of its emission spectrum. Apart from film expansion, the properties of the film were affected only at the water/membrane interface. Proton conduction was prevented only when the surface concentration of the detergent was larger than a critical value. Such an effect could be due either to the disruption in the continuity of the conducting hydrogen-bond network or to an electrostatic repulsion of the protons by the interface.

Lateral conduction of protons along lipid monolayers has been observed by using various methodologies (Teissié & Tocanne, 1990). They can be either direct ones through the observations of the local concentrations of protons at the interface by fluorescence (Teissié et al., 1985), or surface potential (Prats et al., 1986) measurements, or more indirectly through the associated changes in surface pressure (Prats et al., 1987b) and the increase in surface conductance (Sakurai & Kawamura, 1987; Morgan et al., 1988). The process is not at the present time well-characterized on bilayered systems, and conflicting conclusions have been reported by one group where a rather complicated set of equations was solved on a computer in order to explain the experimental fact (Nachliel & Gutman, 1988).

All previous studies were performed on pure lipid systems with only one class of compounds at a time. The main conclusions were that the phenomenon was a diffusion along the interface, presumably through a hydrogen-bond network involving the polar head groups and the hydration layer (Prats et al., 1987a, 1989; Morgan et al., 1988; Teissié et al., 1990). The chemical nature of the polar group of the phospholipid did not play a role, but the state of the monolayer must be liquid (Prats et al., 1987a). In order for diffusion to occur, a decisive property of the monolayer was the need for continuity in the hydrogen-bond network. This was indicated by the collapse of the conduction for very expanded films where

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