Bilayer Partition Coefficients of Alkanols: Predicted Effects of Varying Lipid Composition

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Partition coefficients of short *n*-alkanols between bilayers of different lipid composition (equivalently, the variation in bilayer/water partition coefficients) are calculated as a function of lipid acyl chain length and unsaturation, the strength of lipid headgroup repulsions, and the addition of cholesterol. Predictions are obtained from a statistical thermodynamic approach using a mean field lattice model identical to that used recently to calculate the lateral pressure profile in fluid bilayers. Increasing length, and particularly increasing *cis*-unsaturation of the acyl chains, are predicted to increase the bilayer/water partition coefficients of short-chain alkanols, whereas addition of cholesterol is predicted to have the opposite effect. The magnitude of the shifts are predicted to be significantly larger for lipids with headgroups with little or no repulsions, such as phosphatidylethanolamine, than for more strongly repulsive headgroups such as phosphatidylcholine.

Introduction

The behavior of many integral membrane proteins can be modulated by variations in the composition of the bilayer in which the protein is embedded. In particular, the presence of small amphiphilic solutes such as alkanols is known to have a marked effect on the activity of proteins whose function is associated with a conformational transition, such as rhodopsin,[1] ligand-gated ion channels,[2] etc. The mechanism(s), presently unknown, might be of one of two types. Either the solute could bind directly to site(s) on the protein (either changing its conformational equilibrium or modulating its function in the active state, or both) or it might act by an indirect mechanism, in which its incorporation into the bilayer alters some physical property of the bilayer, which in turn shifts the conformational equilibrium (and thus the activity) of the protein. If the solute acts indirectly, the strength of its influence will depend both on its *specific* activity, i.e., per unit concentration in the bilayer, and on its concentration in the bilayer, linearly in the limit of low membrane concentrations.[3,4] The dependence of specific activities on solute characteristics has been explored in the context of an indirect mechanism based on shifts in the lateral pressure profile.[3,5,6] Unfortunately, measured "dose−response" curves that quantify changes in protein activity report the solute concentration in the aqueous phase, not its concentration in the bilayer. It is therefore important to obtain accurate information on the partitioning of solutes between the aqueous phase and the bilayer. Such partition coefficients have been measured for various solutes, primarily using dimyristoyl phosphatidylcholine (DMPC), dipalmitoyl phosphatidylcholine (DPPC) or egg phosphatidylcholine (PC) bilayers, or erythrocyte ghosts.[7−14] Addition of cholesterol has been shown to decrease the partitioning into DMPC, DPPC, and egg PC bilayers.[14−17] Preliminary results of Rowe et al.[14] and more recently of Rowat and Trandum[18] suggest that bilayer partitioning of short and medium-length alkanols rises with increasing acyl chain length and unsaturation. Recently, Meijer et al.[11] have used an elegant lattice statistical mechanical approach to calculate DMPC/water partition coefficients for a wide range of small solutes, which are found to be in good agreement with unpublished experimental results of Van Lent, as cited therein; however, they have not studied the dependence of partition coefficients on bilayer composition for a given solute.

In the present work, previously developed statistical thermodynamic methods are used to predict effects of changes in bilayer lipid characteristics such as acyl chain length and *cis*-unsaturation, the strength of lipid headgroup repulsions, and addition of cholesterol, on the bilayer/water partition coefficients of short 1-alkanols. First, a simple thermodynamic analysis is used to relate partition coefficients to the standard chemical potentials of the solute in different solvent (bilayer) environments and to the concentration dependence of the bilayer molar free energy. Following a brief summary of the statistical thermodynamic lattice methodology used to calculate the bilayer free energy, predictions of partition coefficients are presented for short alkanols, for a range of lipids.

Thermodynamic Analysis

In the limit of low solute concentration, the chemical potential of a solute varies logarithmically with its concentration, $\mu = \mu^\circ + RT \ln x$. The (arbitrary) choice of concentration units determines the value of the standard chemical potential μ°. For example, the chemical potential of an alkanol at low concentrations in a bulk solvent such as water, μ_w, can be written as

$$\mu_w = \mu_w^\circ + RT \ln x_w \tag{1}$$

For an alkanol solubilized in a lipid bilayer, an analogous expression for its chemical potential μ_b will also be accurate in the limit of sufficiently low alkanol concentration in the bilayer:

$$\mu_b = \mu_b^\circ + RT \ln x_b \tag{2}$$

where x_w and x_b are the alkanol concentrations in the water and bilayer phases, respectively. In this low-concentration limit, the equilibrium partitioning of solute between water (w) and bilayer

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(b) phases is determined from equality of its chemical potential in the two phases: $\mu_b = \mu_w$, or equivalently

$$K_{b,w} = x_b/x_w = e^{-\Delta\mu^\circ/RT} \tag{3a}$$

where $\Delta\mu^\circ = \mu^\circ_b - \mu^\circ_w$.

In general, the standard chemical potential of a solute will depend on the lipid (or lipid mixture) that comprises the bilayer. For bilayers of two different compositions, b1 and b2, we can write

$$K_{b2,b1} = x_{b2}/x_{b1} = e^{-\Delta\mu^\circ/RT} \tag{3b}$$

where $\Delta\mu^\circ = \mu^\circ_{b2} - \mu^\circ_{b1}$; thermodynamic equilibrium requires that $K_{b2,b1} = K_{b2,w}/K_{b1,w}$.

The molar free energy f of a bilayer containing n_{alc} moles of alcohol and n_{lip} moles of lipids (possibly a mixture of different lipids) can be expressed as

$$f = f^* + RT\,[x_b \ln x_b + (1 - x_b)\ln(1 - x_b)] \tag{4}$$

where the mole fraction of alcohol in the bilayer is $x_b = n_{alc}/n$, with $n = n_{alc} + n_{lip}$. The second term in eq 4 represents the ideal molar free energy of mixing of solute with the lipid solvent (at fixed composition, if it is a mixture of lipids). The chemical potential of the alcohol in the bilayer is defined as

$$\mu_b = [\partial(nf)/\partial n_{alc}]_{n_{lip}} = f + (1 - x_b)\,df/dx_b \tag{5}$$

and thus

$$\mu^\circ_b = f^* + (1 - x_b)\,df^*/dx_b \tag{6}$$

To determine the standard chemical potential of an alcohol solute in a bilayer of given lipid composition, as given by eq 6, it is necessary to evaluate the derivative df^*/dx_b. In the present work, a finite difference approximation is performed, by calculating the free energy f_{pure} for the pure bilayer ($x_b = 0$) and calculating f^* at a very dilute concentration of solute, i.e., $0 < x_b \ll 1$. Then eq 6 becomes

$$\mu^\circ_b \approx [f^* - (1 - x_b)f_{pure}]/x_b \tag{7}$$

Equation 7 is used to calculate μ°_b for various lipid bilayer compositions, from which $\Delta\mu^\circ = \mu^\circ_{b2} - \mu^\circ_{b1}$, and thus $K_{b2,b1} = K_{b2,w}/K_{b1,w}$ is determined from eq 3.

Calculational Approach

A mean-field statistical thermodynamic approach is used to calculate the equilibrium properties of the lipid bilayer, using a modified lattice model to describe the chain conformational contributions to the free energy. The approach is identical to that used in recent work[6] to predict pressure profiles for bilayers for a wide range of lipid and lipid/solute compositions. A brief summary of the method and of the approximations is provided here; the interested reader should refer to ref 6 (and the references therein) for details. As with all models, it relies on assumptions and approximations that serve to make the calculations tractable and result in interpretable predictions.

The bilayer is treated as two compact fluid monolayers, in each of which the segments of the acyl chains occupy space at constant bulk density. The distribution of chain segments is described using a cubic lattice model, in which a chain configuration is defined as occupying a particular set of contiguous lattice sites. As in previous work, the boundary between the hydrophilic (headgroup) region and hydrophobic interior of the bilayer is approximated by a sharp planar interface. For the lipids, the junction of each acyl chain with its headgroup is constrained to reside on that plane, so for example, the complexity of the glycerol/carbonyl linkage between the headgroup and the acyl tails in phospholipids is completely ignored, as is the considerable roughness known to be present in the interfacial region. For simplicity of calculation, lipid chains are not allowed to cross the bilayer midplane, i.e., there is no interdigitation between monolayers. The calculations assume the two acyl chains on each lipid are equivalent and act independently. Thus, for example, the chain packing properties of a pure POPC bilayer are calculated identically to that of an equimolar mixture of DPPC and DOPC.

The bilayer free energy contains both entropic and energetic contributions. The configurational entropy of the lipid and solute chains is calculated in mean-field approximation, incorporating bond-correlated excluded volume of chain segments. Three contributions to the internal energy of the bilayer are incorporated: a positive energy of hydrophobic chain segments in contact with water at the aqueous interface, bending stiffness of the acyl chains, and headgroup interactions. (For simplicity, the methylene and terminal methyl groups of the alkanols and of the lipid acyl chains, whether saturated or unsaturated, are taken to have no mixing enthalpy.) At the discrete values of the lipid surface density at which the membrane thickness is an integer multiple of the size of a lattice site, the free energy is minimized with respect to the probability distribution of chain conformations, subject to the constraint of constant bulk density (one chain segment per lattice site) within the hydrophobic core of the bilayer. The calculated free energies are fit to a polynomial in the surface density, the minimum of which determines the equilibrium surface density (i.e., the surface density at zero net lateral pressure) and molar free energy f. This procedure is performed both in the presence and absence of a small mole fraction of solute (typically 1 mol %) to determine f^* and f_{pure}, as described above. Thus the addition of solute occurs at fixed (zero) net lateral pressure, not at fixed surface density.

In unsubstituted, saturated alkanes, the hydrocarbon chain is semiflexible. As in previous work,[6] the statistical weight ω of a chain bend in the cubic lattice model is taken to be $\omega \approx 0.45$, while *cis*-unsaturation results in a strong statistical preference for a bent chain, i.e., $\omega \gg 1$. Calculations are performed for lipids with acyl chains of varying length and unsaturation, including myristoyl (M, 14:0), palmitoyl (P, 16:0), stearoyl (S, 18:0), oleoyl (O, $18{:}1_{\Delta 9}$), and dodecahexaeneoyl (H, $22{:}6_{\Delta 4,7,10,13,16,19}$). Lipid headgroup interactions are modeled through a pairwise additive energy that varies inversely with molecular area, with constant of proportionality u_{hg} that is a measure of the strength of the average repulsion of adjacent headgroups. For PC headgroups, u_{hg} can be estimated[6] to be roughly 1 to 1.5 in units of k_BT (the value depending on temperature; 1.25 has been used in all the calculations presented here), while $u_{hg} \approx 0$ for phosphatidylethanolamine (PE) and for the hydroxyl headgroup in cholesterol and alkanols. Cholesterol, with approximately twice the cross-sectional area of a saturated acyl chain in the all-trans conformation, is modeled as a pair of stiff rods with one end tethered to the interface by the hydroxyl group, with a flexible alkyl chain at the other end of one rod. For 1-alkanol solutes, the interfacial attraction of the hydroxyl group is very strong, so it is assumed always to reside at the aqueous interface.

TABLE 1: Partition Coefficients $K_{b2,b1}$ for 2-Segment Amphiphilic Solutes (as an Approximate Model of Ethanol) between Bilayers b1 and b2, of Different Lipid Composition as Specified[a]

X,Y	$K_{XYPC,DPPC}$	$K_{XYPE,DPPE}$	$K_{XYPE,XYPC}$	$K_{XYPC/chol,XYPC}$	$K_{XYPE/chol,XYPE}$
14:0, 14:0	0.883	0.842	0.701	0.811	0.760
16:0, 16:0	1	1	0.735	0.815	0.786
18:0, 18:0	1.089	1.140	0.769		
16:0, 18:1	1.195	1.340	0.825	0.870	0.867
18:1, 18:1	1.352	1.585	0.862	0.894	0.896
16:0, 22:6	1.553	1.913	0.905	0.930	0.937
22:6, 22:6	1.963	2.489	0.932		

[a] Bilayer lipids are defined by their acyl chains (X,Y)[b] and headgroups (PC or PE). Lipid mixtures with cholesterol are for 10 mol % cholesterol. [b] Acyl chains: myristoyl (14:0), palmitoyl, (16:0), stearoyl (18:0), oleoyl ($18:1_{\Delta 9}$), docosahexaenoyl ($22:6_{\Delta 4,7,10,13,16,19}$). Note that the model treats the two chains on each lipid identically, so calculations for X ≠ Y correspond equally well to a bilayer comprised entirely of XY lipids or to an equimolar mixture of XX and YY (e.g., either pure POPC or an equimolar mix of DOPC and DPPC).

Results

In Table 1 are presented calculated partition coefficients $K_{b2,b1}$ for 2-segment solutes, one segment of which is strongly interfacially active, as a model of a short chain alkanol such as ethanol. Since $K_{b2,b1} = K_{b2,w}/K_{b1,w}$, these results indicate the ratios of bilayer−water partition coefficients for different bilayer composition. Both an increase in chain length and an increase in *cis*-unsaturation are predicted to cause a marked increase in $K_{b,w}$, the effect being significantly more pronounced for PE than for PC headgroups. For a given acyl chain composition, $K_{b,w}$ is predicted to be larger for PC than for PE headgroups, but the difference decreases with increasing unsaturation and chain length. Addition of cholesterol is predicted to lower $K_{b,w}$ significantly, even at 10 mol % cholesterol, the magnitude being by far the largest for saturated chains. The trend continues with increasing cholesterol content; in fact, for those lipids where calculations were performed at cholesterol content as high as 20 mol % (not shown), the drop in $K_{b,w}$ was predicted to occur slightly more rapidly than linearly with increasing cholesterol content.

Calculations of $K_{b2/b1}$ have also been performed for 1-alkanols of varying length, as shown in Figure 1 for representative bilayer (b2) compositions relative to either DPPC or DPPE as the reference bilayer (b1), for alkanols of chain length $n = 2$, 4, 6, and 8. The change in predicted values of $K_{b2/b1}$ with alkanol chain length depends significantly on the bilayers being compared. For example, the magnitude of the drop in partition coefficients upon addition of cholesterol, and the increase with chain unsaturation is predicted to become less significant with increasing alkanol chain length, whereas increased alkanol length accentuates the difference in partition coefficients between PC and PE headgroups.

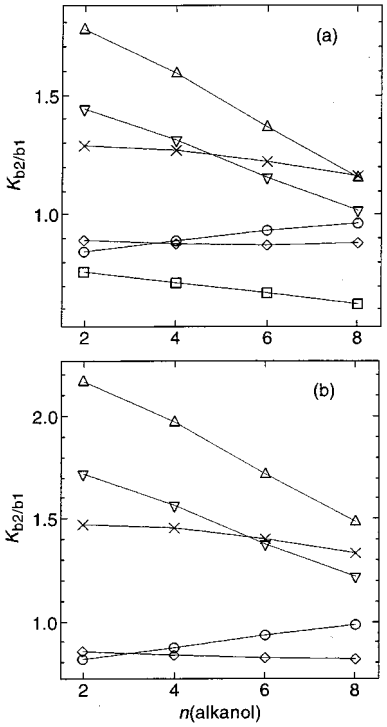

Figure 1. Alkanol partition coefficients $K_{b2/b1}$ between bilayers of different composition as a function of alkanol chain length *n*. (a) b1 (reference bilayer) = DPPC; b2 = DPPE (□), DPPC + 10 mol % cholesterol (○), DOPC (×); PHPC (▽), DHPC (△), DMPC (◇). (b) b1 (reference bilayer) = DPPE; b2 = DPPE + 10 mol % cholesterol (○), DOPE (×); PHPE (▽), DHPE (△), DMPE (◇). Lines are drawn as a guide to the eye.

Discussion

The calculated partition coefficients are based on a statistical thermodynamic approach that involves many approximations, both with respect to the description of the intermolecular interactions and chain conformational states, and with respect to the statistical mechanical (mean field) approach. As a result, predicted numerical values should be viewed as qualitatively but not quantitatively accurate; the trends in the results are expected to be correct. Comparison of the predictions with experimental results is difficult because of the paucity of published results for a given solute for a range of bilayers. Still, the prediction of a drop in alkanol partitioning with increased cholesterol content is consistent with experiment,[14−17] and recent results for different lipid acyl chains and headgroups,[14,18] while preliminary, do indicate increased partitioning with increased lipid acyl chain length and unsaturation, as predicted.

In view of the importance of obtaining bilayer−water partition coefficients, various groups have attempted to develop predictive relationships based largely on the more readily measured octanol−water partition coefficients for a wide range of solutes.[19,20] However, it is important to realize that the variability in the bilayer partition coefficients for different lipid composition may be as large as the difference between octanol−water and bilayer−water coefficients. Clearly, there is a need for more extensive experimental studies of partition coefficients of small solutes, performed over a wide range of lipid composition.

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