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Binding of small alcohols to a lipid bilayer membrane: does the partitioning coefficient express the net affinity?

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Abstract

The total vapor pressures at 26°C of binary (water–alcohol) and ternary (water–alcohol–vesicle) systems were measured for six short chain alcohols. The vesicles were unilamellar dipalmitoyl phosphatidylcholine (DMPC). The data was used to evaluate the effect of vesicles on the chemical potential of alcohols expressed as the preferential binding parameter of the alcohol–lipid interaction, Γ_{23}. This quantity is a thermodynamic (model-free) measure of the net strength of membrane–alcohol interactions. For the smaller investigated alcohols (methanol, ethanol and 1-propanol) Γ_{23} was negative. This is indicative of so-called preferential hydration, a condition where the affinity of the membrane for water is higher than the affinity for the alcohol. For the longer alcohols (1-butanol, 1-pentanol, 1-hexanol) Γ_{23} was positive and increasing with increasing chain length. This demonstrates preferential binding, i.e. enrichment of alcohol in the membrane and a concomitant depletion of the solute in the aqueous bulk. The measured values of Γ_{23} were compared to the number of alcohol–membrane contacts specified by partitioning coefficients from the literature. It was found that for the small alcohols the number of alcohol–membrane contacts is much larger than the number of preferentially bound solutes. This discrepancy, which is theoretically expected in cases of very weak binding, becomes less pronounced with increasing alcohol chain length, and when the partitioning coefficient exceeds approximately 3 on the molal scale (10^2 in mole fraction units) it vanishes. Based on this, relationships between structural and thermodynamic interpretations of membrane partitioning are discussed.

Keywords: Vapor pressure; Preferential binding; Membrane partitioning; Free energy of interaction; Lipid membranes

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1. Introduction

Extensive interest has been devoted to the study of solute partitioning into lipid bilayer membranes. One source of information is the thermodynamic characterization of the process, and the thermodynamic property of most fundamental interest is the free energy of the interaction, which quantifies the net affinity of the solute for the membrane. A range of direct and indirect experimental strategies has been applied to elucidate this property. Direct measurement of the free energy of interaction involves monitoring of the equilibrium distribution between distinguishable phases. This has been done for some membrane-solute systems through the use of, e.g. radiolabeled volatiles in isopiestic methods [1–4] or dialysis equilibrium measurements for non-volatile solutes [5,6]. Free energies and (if the temperature and pressure dependence is known) other thermodynamic properties of solute–membrane interactions can be rigorously derived from this type of data [7,8], and this approach has been extensively used for the characterization of protein–solute interactions (see, e.g. [9–13]). More often, however, reported free energies of solute-membrane interactions are derived from indirect (sometimes denoted 'contact-detecting' [14]) methods. In this approach, the local concentrations of a solute in, respectively, membrane and bulk are estimated. In disperse systems this can be done if an observable (e.g. spectral [15,16] or thermochemical [17–20]) changes upon transfer of the solute between the two states. Alternatively, the local concentrations can be measured analytically after separation of the lipid and aqueous phases by centrifugation [21–23] or filtration [24,25]. When the local concentrations are known, the interaction free energy can be expressed by the standard free energy change, ΔG°, for the process

$$A(\mathrm{aq}) \rightleftarrows A(\mathrm{lip}), \qquad K_\mathrm{p} \tag{1}$$

where A(aq) and A(lip) designates, respectively, the aqueous and membrane partitioned state of the solute, A. The equilibrium constant of Eq. (1) is $K_\mathrm{p} = m_3^{\mathrm{lip}}/m_3^{\mathrm{aq}}$. Here, m indicate mole solute per kg solvent and the sub- and superscript identifies, respectively, the alcohol (component 3) and the solvent (lipid or aqueous). Hereinafter, the three components; water, lipid and alcohol, will be denoted by subscript 1, 2 and 3 in accordance with the suggestion of Scatchard. The standard free energy change, ΔG°, can be derived from the equilibrium (or patititoning) constant through the relationship, $\Delta G^\circ = -RT\ln K_\mathrm{p}$, where R is the gas constant and T the absolute temperature. This direct thermodynamic interpretation of K_p is, however, limited by several assumptions. For example, the chemical potential of A(lip) is specified by ideal solution theory, although size differences between the lipid and solute molecules [26] and a highly non-isotropic distribution of for example alcohols in the membrane [27–30] speak against this assumption [31]. More importantly, from a thermodynamic perspective, the underlying concept of local concentrations is fundamentally problematic. Particularly so when the interaction is weak (K_p is small) [32]. In cases of weak complexation, other factors than those encompassed in Eq. (1) may contribute, and this simple molecular picture cannot realistically describe membrane-solute interactions. This is an example of the general discrepancy between thermodynamic and structural or stoichiometric interpretations of weak association processes, which has been discussed extensively for protein–solute systems [8,14,33].

A thermodynamic treatment of weak association processes can be achieved through the use of preferential binding parameters. Hence, the preferential binding parameter of the alcohol-lipid interaction, Γ_{23}, is defined [14,33]

$$\Gamma_{23} = \left(\frac{\partial m_3}{\partial m_2}\right)_{T,P,\mu_3} \tag{2}$$

where the molalities (m) of alcohol (3) and lipid (2) are total concentrations in mole per kg water. T,P and μ_3 are, respectively, temperature, pressure and the chemical potential of the solute, A. Thus, Γ_{23} is the number of A molecules, which has to be added to reestablish μ_3 following the addition of one lipid molecule. Unlike Eq. (1) this

approach does not rely on the designation of local compartments of the system, and the free energy of interaction and other thermodynamic parameters can be derived directly from Γ_{23}. In cases of strong binding or partitioning the meaning of Γ_{23} and K_p will be tantamount [cf. Eq. (5)]. At the other extreme, Γ_{23} can be zero or negative and thus, unlike K_p, rationalize unfavorable ('repulsive') membrane-solute interactions. This latter condition is generally referred to as preferential exclusion of the solute (or preferential hydration of the biopolymer) [14,34,35]. It implies that the membrane-domain is enriched in water while the concentration of the solute is reduced compared to the aqueous bulk. It does not infer the absence of alcohol–solute contacts as defined by Eq. (1), and there is no explicit relationship between K_p and Γ_{23}.

In the present work we used vapor pressure measurements to determine Γ_{23} for the interaction of large unilamellar vesicles of dipalmitoyl phosphatidylcholine (DMPC) in the fluid phase[1] with the normal alkane-mono-ols from methanol to 1-hexanol. DMPC membranes were found to be preferentially hydrated in solutions of the smaller of these alcohols, while the longer were shown to bind preferentially. The problems of comparing partitioning coefficients and preferential binding parameters discussed above were shown to be important for K_p values lower than 3 in molal units.

2. Experimental

Alcohols were purchased from Merck (Darmstad, Germany: 1-propanol > 99.8%, 1-butanol > 99.5%, 1-pentanol 98.5% and 1-dodecanol > 98%), Sigma (St. Louis, USA: ethanol > 99.8%), Fluka (Buchs, Switzerland: 1-hexanol > 99%) and May & Baker (Dagenham, UK: methanol 99.8%) and used without further purification. Dimyristoyl phophatidylcholine, DMPC (> 99% Avanti Polar Lipids, Birmingham, USA) was suspended in milliQ water and allowed to hydrate for approximately 1 h at 40°C. Subsequently large unilamellar vesicles were produced by extrusion [40] through 0.1-μm polycarbonate filters. The concentration of lipid in the extruded samples was determined gravimetrically by drying several 50-μl aliquots.

Total vapor pressures of two component (water + alcohol) and three component (water + alcohol + DMPC) systems were measured by equipment designed and build in our laboratory. The principles of the apparatus, which is a 'second generation' of the one described previously [41], are briefly presented below. Further technical details will be given in a forthcoming note.

An aliquot of approximately 3 ml (water or 90 mM DMPC) quantified gravimetrically was initially transferred to the sample cell (10-ml glass bulb) and extensively degassed. The pressure difference between this sample and a reference (pure water), placed close together in a thermostat bath, was measured by high precision capacitance manometers (model 616A, MKS Instruments, Andover, USA). The pressure difference between pure water and the lipid suspension was too low to be detectable. The sample was subsequently titrated with one of the six investigated alcohols without breaking the vacuum or changing the amounts of water or lipid in the sample cell. To do so, we utilized a system of glass flasks on a stainless steel manifold connected through bellow valves (Nupro Willoughby, USA) to the cells and manometers. Initially, degassed, liquid alcohol contained in a flask on the manifold, was allowed to expand into a known, previously evacuated volume (71.1 ml or 538.7 ml in this work) to reach a pressure (typically 80 mbar) well above the vapor pressure of the sample cell. The pressure in this 'charging-compartment' was measured by a separate high precision manometer (MKS 615A). The alcohol gas was then allowed to expand from the charging-compartment into the sample cell

[1] The main transition temperature, T_m, of DMPC is 24°C. Small amounts of alcohols decrease this temperature. Higher alcohol concentrations may stabilize the gel phase and thus increase T_m. This effect is particularly strong for small alcohols and long acyl chain lengths of the lipids. For the relatively short (C14) acyl chain of DMPC and the concentrations of alcohols used in this study it is reasonable to assume that the vesicles are in the fluid state throughout the titration trials [36–39].

and the amount of gas transferred was quantified by the ideal gas law from the temperature, charging volume and pressure change. To avoid condensation, the charging compartments and manometers were in an air bath at 102 ± 0.2°C. Hence, the sample was always much colder than the rest of the system. The pressure in the sample cell, relative to water (in the reference), was monitored until a stable (equilibrium) value was reached. The equilibrium time was 15–20 min for the smaller and several hours for the longer alcohols. Through a series of such alcohol titrations from the gas phase, the dependence of the total pressure on the alcohol concentration could be established for both binary (water + alcohol) and ternary (water + alcohols + DMPC vesicles) systems. The experimental temperature was 26.0 ± 0.01°C.

3. Results and discussion

Fig. 1a,b shows raw data from the vapor pressure measurements. The plots illustrate the pres-

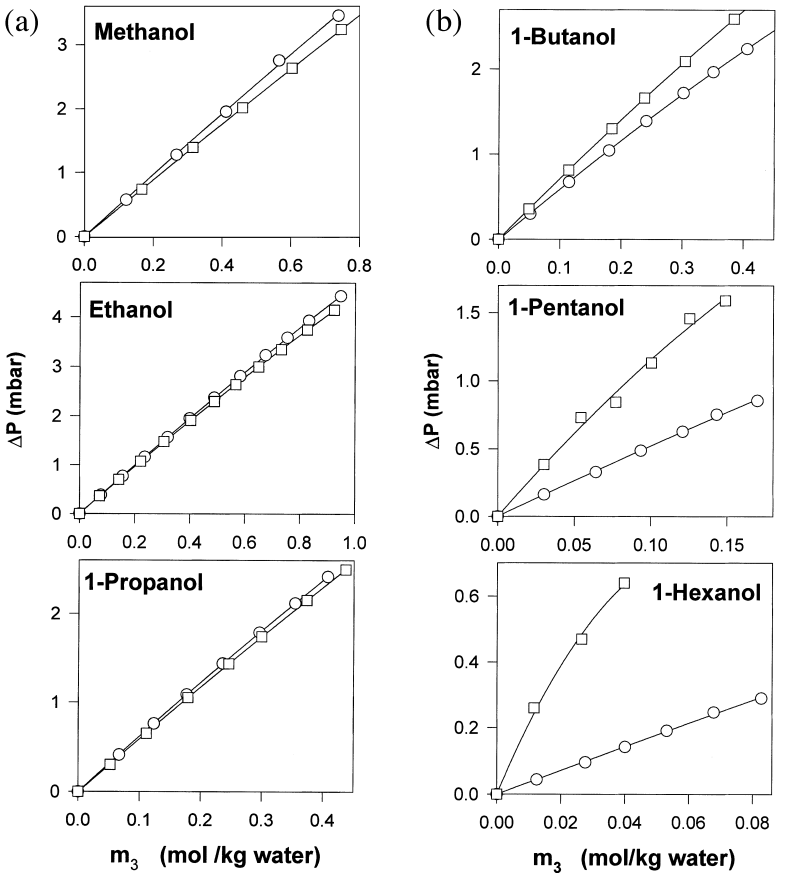

Fig. 1. a: Increase in total pressure ($\Delta P = P_{cell} - P_1^*$, where P_{cell} is the vapor pressure of the sample and P_1^* is the vapor pressure of pure water, 33.60 mbar) as a function of the alcohol concentration, m_3, in mol/kg water. Squares indicate trials with binary (water + alcohol) mixtures and circles refer to suspensions of unilamellar DMPC vesicles. The points are the raw data from the pressure measurements and the lines are quadratic fits used to compare the two experiments at a fixed value of ΔP (see Fig. 3). The DMPC concentrations were methanol: 6.35% (w/w), ethanol: 6.55% and 1-propanol: 6.02%. b Increase in the total pressure, ΔP, as a function of the alcohol concentration. Symbols are as in Fig. 1a. The DMPC concentrations are 1-butanol: 6.02% (w/w), 1-pentanol 6.02% and 1-hexanol 5.47%.

sure increase, ΔP, in the sample cell as a function of the total concentration of the alcohol. Squares in Fig. 1 indicate vapor pressures of binary (alcohol–water) systems while circles refer to trials with DMPC suspensions. The concentration of DMPC was approximately 6% w/w (90 mM) in all cases; precise values are given in the legends of Fig. 1a,b. The curves are polynomials fitted to the data points to allow estimation of the difference in vapor pressure at a certain composition. The total pressure of the sample is the sum of ΔP and the (reference) pressure of water at 26°C (33.60 mbar).

The partial pressures of alcohol and water in the binary systems were calculated by numerical integration of the Gibbs–Duhem equation [42,43]. We applied a previously established procedure [41,44] in which the gas phase is considered ideal and a small correction for the amounts of alcohol and water vapor in the dead space (i.e. the volume of the vapor above the liquid sample) of the system (70 ml) is introduced in the calculation of the composition of the liquid phase.

From the partial pressure of the alcohol, P_3, and the vapor pressure of the pure alcohol at the same temperature, P_3^* (found in a separate measurement) the activity coefficient, γ_3, of the alcohol in binary aqueous solution could be calculated, $\gamma_3 = P_3/x_3\ P_3^*$, where x_3 is the mole fraction of the alcohol. In the most dilute range we found activity coefficients of 1.6, 3.8, 14.0, 54, 250 and 1150 for, respectively, methanol, ethanol, 1-propanol, 1-butanol, 1-pentanol and 1-hexanol. This is in accordance ($\pm 8\%$) with the data at 25°C calculated from $\Delta\mu^\circ$ values in [45] (table 4.2) using $\gamma_3 = \exp\{\Delta\mu^\circ/RT\}$.

The partial pressures of ternary solutions can also be determined by numerical treatment of the Gibbs–Duhem equation [46] and other approaches [47]. In this work, however, we analyze the vapor pressure data by assuming that the vesicles constitute a separate phase. While the general validity of this assumption may be debatable, the lack of a detectable vapor pressure depression in vesicle suspensions found here suggests that interactions of vesicles with the mother liquid contributes negligibly to the properties of the system compared to effects of other intermolecular interactions in solution. Thus, we calculate Γ_{23} directly from the difference between the two curves in a panel of Fig. 1 as described below.

The molal concentration on the abscissa of Fig. 1, m_3, is

$$m_3 = \frac{n_3}{ms_1} \tag{3}$$

where n_3 designates the number of moles of alcohol, and ms_1 is the mass of water. This expression for m_3 is used for both types of systems [without introducing the lipid in the denominator of Eq. (3) in samples with vesicles]. As a result, the two curves in a panel of Fig. 1 will be superimposed if the lipid phase is impenetrable to the alcohol and the alcohol does not interact favorably with the membrane interface. If, on the other hand, the membrane absorbs and/or adsorbs alcohol, the activity of the solute in the aqueous phase will be lowered and the DMPC trials (circles) will show a lower vapor pressure than the binary mixture at a given value of m_3. This latter situation is observed for 1-butanol, 1-pentanol and 1-hexanol. For the three shorter alcohols, on the other hand, the presence of DMPC vesicles acts to increase the total vapor pressure. This observation is not immediately compatible with the molecular picture in Eq. (1); the weakest interaction that can be rationalized through Eq. (1) is $K_p = 0$ which would imply superimposed curves in Fig. 1. To illustrate this further, we made an additional titration of 1-propanol into a sample containing water and 1-dodecanol (i.e. a bulk oil phase instead of DMPC vesicles). The results in Fig. 2 show that while DMPC vesicles act to increase the total pressure of the sample, 1-dodecanol has the opposite effect. The dodecanol/water partitioning coefficient of propanol determined directly from the difference between the two curves (circles and triangles) in Fig. 2 was 14.1 (mole fraction units) in the most dilute range. If we assume ideal behavior of dodecanol/propanol mixtures and neglect the mutual solubility of water and dodecanol, this means that the activity coefficient of propanol in water is 14.1. This number coincides

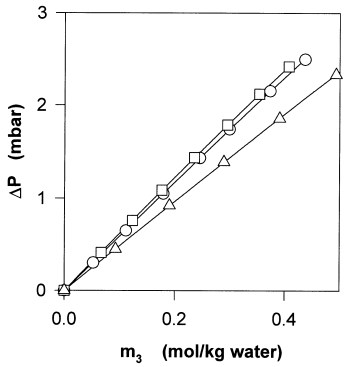

Fig. 2. Increase in total pressure, ΔP, as a function of the molal concentration of 1-propanol. The three solvents are water (circles), water with 6.02% (w/w) unilamellar DMPC vesicles (squares) and water with 12.7% (w/w) 1-dodecanol (triangles). It appears that ΔP is increased by vesicles but decreased by the presence of a drop of bulk oil. See text for details.

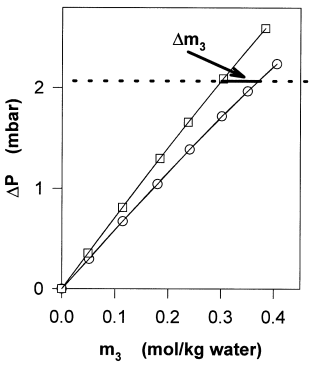

Fig. 3. Illustration of the method used to find Γ_{23} from the vapor pressure measurements. Since ΔP in a two-phase system defines a certain value of the chemical potential of the alcohol, μ_3, the horizontal distance between the two curves specifies the number of moles of alcohol needed to reestablish μ_3 upon addition of vesicles. In this example (1-butanol from Fig. 1b) addition of vesicles (6.02% (w/w) or 0.095 mol/(kg water)) to a binary aqueous solution with $m_3 = 0.3$ mol/(kg water) lowers ΔP from approximately 2.1 to 1.7 mbar. Subsequent addition of butanol increases the pressure along the curve (circles) until the total pressure (and hence μ_3) has been raised to the level of the dotted line. The additional butanol is Δm_3. In this example $\Delta m_3 = 0.065$ mol/(kg water). Hence, $\Gamma_{23} = 0.065/0.095$.

well with the activity coefficient determined from the gas–liquid equilibrium in the binary system (14.0). This result supports the validity of the experimental method, and it underscores the differences between the modes of interactions of alcohols with a membrane and a bulk organic phase.

Based on the assumption that the vesicles constitute a phase separate from the mother liquid, the latter is simply a binary aqueous solution of the alcohol. It follows that the partial pressures, and hence the total pressure, are unique at a given composition.[2] Conversely, at a given total pressure, the composition of the aqueous phase (and hence chemical potential of alcohol in the two equilibrated phases) is uniquely specified. The value of Γ_{23} is then directly calculated from the shift in alcohol molality, Δm_3 defined in Fig. 3, upon an increase in the lipid concentration, Δm_2 ($\Gamma_{23} \approx \Delta m_3/\Delta m_2$ at constant total pressure and small values of Δm_2).

[2] This is only true when the total pressure increases monotonously with alcohol concentration, i.e. until the azeotropic composition is reached at alcohol concentrations well above the ones used here.

The dependence of Γ_{23} on the alcohol concentration determined from the smooth curves in Fig. 1 is illustrated in Fig. 4 (solid lines) for all six alcohols. It appears that the slope of $\Gamma_{23}(m_3)$ increases with the alcohol chain length; it is negative for methanol and ethanol and strongly positive for 1-pentanol and 1-hexanol. This is in accordance with the well-established view that the affinity for lipid membranes increases with the hydrophobicity of alcohols. In contrast to conclusions based on indirectly determined free energies, however, Fig. 4 shows that the net interaction of vesicles with methanol, ethanol and 1-propanol is unfavorable. Thus, for methanol at a concentration of $m_3 = 200$ mmol/(kg water) it is found that $\Gamma_{23} = -0.2$. This means that addition of one DMPC vesicle (10^5 DMPC molecules) requires the removal of 2×10^4 molecules of methanol from the system to reestablish its chemical potential.

To systematically compare the present data with partitioning coefficients we introduce a function

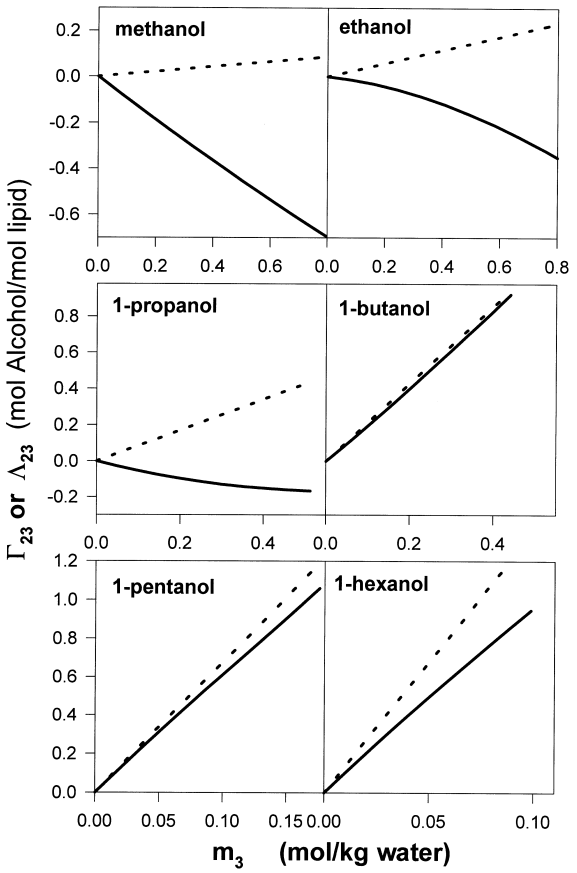

Fig. 4. The preferential binding parameter, Γ_{23} (solid lines) and the number of membrane-alcohol contacts specified by the partitioning coefficient, Λ_{23} (dotted lines) plotted as a function of the alcohol concentration. Γ_{23} is calculated from the horizontal difference between the smooth curves in Fig. 1 as illustrated in Fig. 3. Λ_{23} is determined from Eq. (7) and literature values of the membrane-water partitioning coefficient.

Λ_{23}

$$\Lambda_{23} = \frac{dn_3^{lip}}{dn_2} \tag{4}$$

where n_3^{lip} is the number of moles of alcohol in the membrane as defined by the partitioning coefficient and n_2 is the number of moles of DMPC. Thus, Λ_{23} is the increase in the number of partitioned alcohol molecules following the addition of one DMPC molecule to the system. In that sense Λ_{23} is related to Γ_{23}. However, there is the fundamental difference that Λ_{23} is a non-thermodynamic function quantifying membrane-alcohol contacts according to the scheme of Eq. (1) while Γ_{23} reflects the preferential interaction devoid of an underlying molecular picture. Coincidence of Λ_{23} and Γ_{23} comply with the simple picture that partitioned alcohol molecules (as defined by K_p) no longer contribute to the chemical potential, μ_3, in the bulk. If, on the other hand, $\Lambda_{23} > \Gamma_{23}$ the number of alcohol–membrane contacts exceeds the preferential binding parameter.

To estimate the value of Λ_{23} we utilize the definition of the partitioning coefficient in molal units $K_p = m_3^{lip}/m_3^{aq} = (n_3^{lip}\ ms_1)/(n_3^{aq}\ ms_2)$, where again m, n and ms indicate, respectively, molality, number of moles and mass. Insertion of the mass balance for the alcohol, $n_3^{lip} = N_3\text{-}n_3^{aq}$ and isolation of n_3^{lip} yields

$$n_3^{lip} = \frac{K_p ms_2/ms_1}{1 + K_p ms_2/ms_1} N_3$$

Hence, the dependence of the number of partitioned alcohol molecules, n_3^{lip} on the amount of lipid (in moles), $n_2 = ms_2/M_2$, where M designates molar mass, can be expressed

$$n_3^{lip} = \frac{\beta n_2}{1 + \beta n_2} N_3 \tag{5}$$

where for convenience the term $K_p M_2/ms_1$, which is independent of n_2, is written β. Combination of Eq. (4) and Eq. (5) gives

$$\Lambda_{23} = \frac{\beta}{(1 + \beta n_2)^2} N_3 \tag{6}$$

Λ_{23} was calculated from Eq. (6) and literature values of K_p [17,48,49]. The values are plotted in Fig. 4 (dashed lines) against the calculated bulk molality of the alcohol, $m_3^{aq} = (N_3 - n_3^{lip})/ms_1$. In accordance with the qualitative arguments above, comparison of the two functions for the three smaller alcohols show that the number of alcohol–membrane contacts defined by K_p is much larger than the number of preferentially bound

solutes. The discrepancy decreases with increasing alcohol chain length and for 1-butanol there is an almost perfect match between Λ_{23} and Γ_{23}. For 1-pentanol and 1-hexanol Γ_{23} is approximately 20% larger than $\Lambda_{23,}$ but since this difference is rather small compared to the disparity of reported K_p values (see, e.g. [50,51]) we conclude that Λ_{23} and Γ_{23} are similar for the three longer alcohols. This suggests that the partitioning model in Eq. (1) provides a sound outset for a thermodynamic treatment when K_p is larger than approximately 3 in molal units (approximately 100 in mole fraction units). Conversely, in solutions of the smaller alcohols, DMPC membranes are preferentially hydrated, and this situation cannot be adequately described by Eq. (1), or any other molecular picture that does not explicitly account for a role of water. It should be noted that discrepancy of Λ_{23} and Γ_{23} is not per se indicative of deficiencies in the experimental data underlying these two quantities. Indeed, it is expected that the number of alcohol-membrane contacts will be larger than Γ_{23} in cases of weak interactions. Random distribution of alcohol in this situation, with a higher bulk-to-membrane concentration ratio, will lead to a significant number of membrane-alcohol contacts, which will contribute to Λ_{23} but not to Γ_{23}.

An interesting comparison of the data in Fig. 4 can be brought about by converting the abscissa from concentration units to activities. This can be readily done since $\mu_3(\Delta P)$ is known from the binary data in Fig. 1. The results of this conversion are illustrated in Fig. 5 showing Λ_{23} (panel a) and Γ_{23} (panel b) as a function of the activity of the alcohol, a_3. As above a_3 is the activity on the mole fraction scale with respect to the pure liquid standard state. Hence, $a_3 = P_3/P_3^*$ and it specifies the net propensity of the alcohol to escape to another phase. This means that the data in Fig. 5 are 'normalized' with respect to the hydrophobic drive on the aqueous alcohol, and the plots thus emphasize the intrinsic or direct effects of alcohol–membrane interaction. A noticeable result in Fig. 5 is that all curves in panel A are practically superimposed. This suggests that when normalized to the aqueous activity, the (intrinsic) propensity to partition into

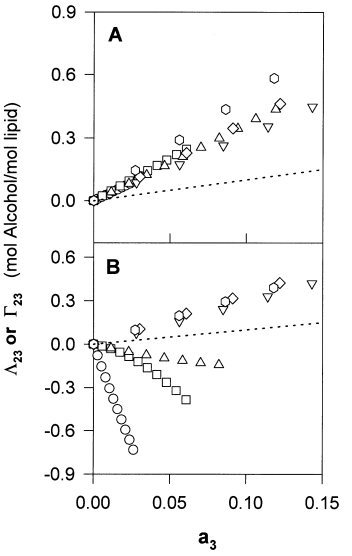

Fig. 5. The same data as in Fig. 4 plotted as a function of the alcohol activity (rather than concentration). The activities were calculated from the activity coefficients found in the binary solutions (Fig. 1). Panel a shows the number of alcohol contacts, Λ_{23}, while panel b shows the preferential binding parameter Γ_{23}. The symbols (in both panels) indicate: circles: methanol, squares: ethanol, up triangles: 1-propanol, down triangles: 1-butanol, diamonds: 1-pentanol and hexagons: 1-hexanol.

DMPC is the same for all the investigated alcohols, which cover a range of three orders of magnitude of activity coefficients. Furthermore, it appears that this propensity is somewhat larger than that of bulk oil partitioning [45] indicated by the dotted line in the figure. In a solution with $a_3 = 0.01$, for example, the concentration of alcohol (any of the six) in DMPC membranes is 1 alcohol molecule for each 25–30 DMPC. This is in contrast to the ratio of 1:100, which is found in bulk partitioning experiments (and expected for membrane partitioning if the *A*(lip)-state of Eq. (1) was an ideal solution). A tentative conclusion of Fig. 5a is that the driving force of alcohol partitioning is predominantly unfavorable hydrophobic interactions in the aqueous bulk. This effect is supplemented by a smaller attractive component, possibly polar interactions involving the –OH group, which is similar for all the alcohols.

For the three longer alcohols the plots of $\Gamma_{23}(a_3)$ (Fig. 5a) are effectively superimposed lines with a slope of 3–4, i.e. similar to that of the $\Lambda_{23}(a_3)$ data. This supports the above conclusion on driving forces for membrane–alcohol association. Thus, the affinity of these alcohols for the membrane is specified by their activity and slightly higher than the affinity for a bulk organic phase (dotted line, data from dodecanol measurement, Fig. 2). For the shorter alcohols, Fig. 5b stresses the difference between preferential binding and partitioning. It appears that the (intrinsic) membrane-alcohol interaction becomes increasingly unfavorable the shorter the alcohol acyl chain. This observation is interesting in comparison with the invariant $\Lambda_{23}(a_3)$ values for all six alcohols (Fig. 5a). It might be expected that alcohol–membrane contacts became less frequent (the slope of $\Lambda_{23}(a_3)$ in Fig. 5a decreased) in solutions of methanol and ethanol, where the membranes are preferentially hydrated. The lack of this correlation between Λ_{23} and Γ_{23} suggests that the probability of small alcohols to occupy a site in (or at) the membrane is a complex function of the concentration and activity of alcohol in the aqueous phase as well as the concentration of water in the membrane (see closing remarks).

So far Γ_{23} has been used to discuss the 'free energy of interaction'. The data in Fig. 5b readily allows estimation of this quantity in more conventional terms. Hence [12,33]

$$\Delta\mu_3 = -RT\int_0^{a_3}\frac{\Gamma_{23}}{a_3}da_3 \qquad (7)$$

Where $\Delta\mu_3$ is the free energy of interaction specified by the change in the chemical potential of the alcohol upon transfer from water to lipid suspension. Hence, if Fig. 5b is re-plotted to show Γ_{23}/a_3 the area under the curve will be $-\Delta\mu_3/RT$. If again $a_3 = 0.01$ is used as an example, interaction free energies calculated from Eq. (7) are, respectively, 600, 100 and 60 J/mol for methanol, ethanol and 1-propanol. For the three longer alcohols the free energies of interaction were -70 J/mol (1-butanol) and -90 J/mol (1-pentanol and 1-hexanol). The constant value for the longer alcohols may reflect the intrinsic attractive contribution mentioned above. If instead $\Delta\mu_3$ is compared at a fixed concentration of alcohol the results are strongly dominated by the hydrophobic drive on the alcohols. At a mole fraction $x_3 = 10^{-4}$, for example (a concentration low enough for all alcohols to be soluble), $\Delta\mu_3$ decreases from practically zero ($+5$ to $+10$ J/mol) for methanol and ethanol to -0.25 and -1.0 kJ/mol for, respectively, 1-pentanol and 1-hexanol.

4. Closing remarks

The vapor pressure data show that unilamellar vesicles of DMPC in the fluid phase act to increase the chemical potential of aqueous methanol, ethanol and 1-propanol. In the language used for protein–cosolvent systems this implies that the membranes are preferentially hydrated in these solvents. The interpretation of this observation is not unambiguous for a membrane system. Since small alcohols readily permeate phospholipid bilayer membranes, some fraction of the alcohol molecules must be out of the aqueous phase. This 'dilution' of alcohol will decrease ΔP and thus generate a positive contribution to Γ_{23}. The observation of negative values suggests either that this effect is more than balanced out by unfavorable interactions at the membrane water interface, or that the penetration of alcohols into the membrane has a concomitant influx of water. In the latter case, the ratio of the water and alcohol partitioning would determine the sign of Γ_{23}. Thus, if an increase in the average water content of the membrane surpassed the alcohol partitioning, ΔP would be positive (Γ_{23} negative). Some support for this interpretation comes from spectroscopic, kinetic and thermodynamic data, which suggest an increase in the water content of bilayer membranes resulting from partitioning of alcohols and other solutes [52–55].

For the three longer alcohols on the other hand, the present analysis of the preferential binding parameters supports the validity of the picture of Eq. (1).

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