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Packing properties of 1-alkanols and alkanes in a phospholipid membrane

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Abstract

We have used vibrating tube densitometry to investigate the packing properties of four alkanes and a homologous series of ten alcohols in fluid-phase membranes of dimyristoyl phosphatidylcholine (DMPC). It was found that the volume change of transferring these compounds from their pure states into the membrane, ΔV_m(pure → mem), was positive for small (C4–C6) 1-alkanols while it was negative for larger alcohols and all alkanes. The magnitude of ΔV_m(pure → mem) ranged from about +4 cm^3/mol for alcohols with an alkyl chain about half the length of the fatty acids of DMPC, to −10 to −15 cm^3/mol for the alkanes and long chain alcohols.

On the basis of these observations, previously published information on the structure of the membrane–solute complexes and the free volume properties of (pure) phospholipid membranes, we suggest that two effects dominate the packing properties of hydrophobic solutes in DMPC. First, perturbation of the tightly packed interfacial zone around the ester bonds and first few methylene groups of DMPC brings about a positive contribution to ΔV_m(pure → mem). This effect dominates the volume behavior for alcohols like 1-butanol, 1-pentanol and 1-hexanol. More hydrophobic solutes penetrate into the membrane core, which is loosely packed. In this region, they partially occupy interstitial (or free-) volume, which bring about a denser molecular packing and generate a negative contribution to ΔV_m(pure → mem).

Keywords: Densitometry; Excess volume; Membrane partitioning; DMPC; Lipid packing; *n*-alcohols; Alkanes

1. Introduction

The properties of "foreign molecules" or solutes in lipid membranes continue to attract considerable research interest. This relies on the relevance of membrane–solute complexes as models for a range of processes of biochemical and medical interest. Some examples include passive transport through biomembranes, the mechanism and pharmaco-kinetics of anesthetics and other drugs and the lodging of peripheral membrane proteins by insertion of hydrophobic anchors. Investigations of membrane–solute complexes also underlie the rational use of lipid membranes in biotechnology, for example as drug carriers or hosts for reconstituted membrane proteins. One successful approach to elucidate membrane–solute interrelationships has been the combined interpretation of structural and thermodynamic data. This is particularly true in cases where the thermodynamic work involved systematic, comparative investigations of structurally related solutes. Examples of this type of work include studies of the changes in free energy [1–3] or enthalpy [3–5] of membrane partitioning for homologous series of solutes. Although some studies have addressed the volumetric behavior of membrane–solute complexes [6–16], systematic information relating molecular packing properties and the chemical structure of the solute is not yet available. This type of information appears to be of interest due to the relationship between volume and structure, which is more readily established than structural interpretations of other thermodynamic functions such as enthalpy changes. Combined interpretation of information on membrane thickness and volume, for example, directly elucidates the scaling between lateral and normal expansion and the possible decoupling of the two [17].

In this work, we analyze a number of structurally related hydrophobic solutes, and suggest some relationships between packing properties in the doped membranes and the chemical structure of the solute.

2. Experimental

Due to the pronounced differences in water solubility of the investigated solutes, two strategies of sample preparation were adopted.

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2.1. Method 1

The smaller alcohols have high to moderate solubility in water and the partitioning process can be readily equilibrated by simply adding the pure alcohol to a lipid suspension. To this end we suspended DMPC in freshly prepared MilliQ water and left the sample ~10 °C above the main transition temperature (which is 23.9 °C) for an hour. To facilitate hydration, the sample was repeatedly shaken and exposed to light ultrasound treatments (Cole Palmer 8890 ultrasonic bath). The latter also promoted the break-up of large lipid aggregates and hence effectively prevented sedimentation in the measuring tube during the few minutes the lipid sample was in the densitometer (see below). The concentration of DMPC, which was about 4% w/w in all trials, was measured gravimetrically to within ±0.05% w/w as described earlier [18].

The desired amount of the pure solute was transferred with a pipette into an empty glass vial and quantified gravimetrically. Immediately after, 1.3 ml lipid suspension was added to the vial, which was then weighed and hermetically closed. The lipid/alcohol system was allowed to equilibrate for 24 h under gentle agitation in a bath at 45 °C. The lack of evaporative loss during the equilibration was ensured by re-weighing. The samples were then placed in an air-bath at 40 °C, from which they were taken (and vortexed) just before the density measurement. Thermostatting the sample above the experimental temperature was found to strongly reduce problems with bubble formation in the densitometer. The samples were prepared so that the solute concentration in the membrane covered the range of 3–10 DMPC molecules per solute molecule.

For some solutes method 1 was also used in a modified version in which the hydrated lipid suspension was extruded into 100 nm unilamellar vesicles using a commercial equipment (Lipex Biomembranes Inc. Vancouver BC). Concentration determination and solute equilibration of the extruded vesicles was conducted as described above for the multilamellar samples.

2.2. Method 2

For solutes which are practically insoluble in water (i.e. the alkanes) we found that method 1 required very long equilibration times (>2 days). To validate results derived from these extended equilibration times, we also tested alkane-samples prepared by an alternative procedure. A suspension of multilamellar DMPC was prepared as above. Aliquots of 1.3 ml of the suspension were transferred into glass vials, which were then freeze-dried for 48 h. The dry lipid (in the vial) was weighed and the liquid solute was added and quantified gravimetrically. After closing the vial carefully, it was allowed to equilibrate for 2 days at 50 °C. This makes the solute vapor adsorb evenly as it solvates the dry lipid matrix. Several control vials without solute were also exposed to 50 °C. The samples (cooled to room temperature) were added 1.3 ml freshly prepared MilliQ water (quantified precisely by weight). The samples were hydrated as described in method 1 and kept at room temperature overnight. To minimize the risk of bubble formation in the densitometer, they were placed in the 40 °C several hours before the density measurements.

2.3. Densitometry

The glass vials were opened immediately before use and the sample was loaded into the densitometer (DMA 602, Anton Parr, Grass Austria) with a 2 ml syringe. The vibration frequency was read at 30 s intervals over 4–6 min until a constant reading signified thermal equilibrium. The sample was then removed and the densitometer was rinsed thoroughly with pure methanol an dried for ~5 min before loading the next sample. The densitometer was calibrated against air and pure water several times a day. The experimental temperature was 30 °C and controlled to within ±0.010 °C. The absolute value was confirmed against a mercury thermometer with a calibration traceable to the Force Institute (Copenhagen, Denmark). During the measurements, temperature variations were recorded by a NRT thermistor placed next to the vibrating tube in the densitometer. The experimental scatter on repeated measurements on the same sample corresponded to about 5×10^{-6} cm^3/g.

2.4. Scanning calorimetry

The phase behavior of phospholipid–alkanol systems has been thoroughly investigated for smaller alcohols (less than ~10 carbon atoms) (see e.g. [3,14,19–21]). This work collectively indicates that the systems investigated here will be in the fluid (L_α) phase. For more hydrophobic solutes (alkenes and alkanols with ten or more carbons) the main transition temperature, T_m, may increase with the solute concentration, and we therefore checked their phase behavior by DSC. Hence, following the densitometric measurement, 25 μl of the sample was diluted with 2000 μl water, mixed and transferred to the cell of a MC2 DSC (Microcal Amherst MA). The sample was then heated from 10 to 60 °C at a rate of 1 °C/min, and the gel-to-liquid phase transition identified by the endothermic peak. Since the partitioning coefficients of the solutes investigated by DSC are very large ($>10^5$), the membrane concentration of the solute changes only negligible as a result of the dilution of the DSC samples (cf. Eq. (7)).

2.5. Materials

Dimyristoyl Phosphatidylcholine (DMPC, >99%) was purchased as powder (Avanti Polar Lipids Alabaster, AL). The solutes were *c*-hexane (>99.5%, Fluka, Buchs Switzerland), *n*-octane (>99.5%, Fluka) *n*-decane (>98%, Fluka) *n*-dodecane (>98, Fluka), 1-propanol (>99.8%, Fluka), 1-butanol (>99.5%, Merck, Hohenbrunn, Germany), 1-pentanol (>99%, Merck), 1-hexanol (>99%, Fluka), 1-heptanol (>99%, Merck), 1-octanol (>99.5%, Fluka), 1-nonanol (>98%, Merck), 1-decanol (>99.5%, Fluka), 1-undecanol (>98, Merck) and 1-dodecanol (>99.5% Fluka).

3. Data analysis

3.1. Water soluble solutes

To illustrate membrane-solute packing effects, we calculated the excess specific volume of the samples. This function illustrates the difference between the specific volume of a mixture and the volume of its constituent components in their standard state. In other words, it quantifies the volume change associated with the reorganization of the mixing process. In the present experimental protocol, it is advantageous to consider the (lipid–water–solute) system as a pseudo two-component mixture consisting of lipid suspension and solute. This follows from the fact that the lipid suspension used in each experimental series is taken from the same stock solution. The specific volume of this stock solution V_{stock} is known with high precision (from triplicate measurements in each series) and the excess specific volume of the three component system, ${}^3V^{\text{E}}$, can thus be calculated (without introducing the concentration of lipid)

$$ {}^3V^E = {}^3V - wV_S^* - (1-w)V_{\text{stock}} \quad (1)$$

In Eq. (1), ^{3}V is the measured specific volume of the three component system, V^*_{S} is the specific volume of the pure (liquid) solute measured in a separate experiment and w is the weight fraction of solute

$$ w = m_{\text{s}}/(m_{\text{s}} + m_{\text{stock}}) \quad (2)$$

where m is mass and subscripts S and stock identifies respectively solute and (solute-free) lipid stock solution (m_{stock} is the sum of the masses of water and lipid).

For solutes with a non-negligible water solubility, ${}^3V^{\text{E}}$ reflects both effects on the membrane and the aqueous bulk. To single out membrane packing properties, we analyzed binary mixtures of alcohols. The (total) specific excess volume of the binary mixtures, ${}^2V^{\text{E}}$, at different alcohol concentrations was calculated as (cf. Eq. (1))

$$ {}^2V^{\text{E}} = {}^2V - wV_S^* - (1-w)V_{\text{H2O}}^* \quad (3)$$

where ${}^2V$ is the measured specific volume and V^*_{H2O} is the specific volume of pure water. To compare this data with the literature, we calculated the excess partial specific volume of the alcohol $V^{\text{E}}(S)$ using the standard "method of intercepts" [22].

$$ V^{\text{E}}(S) = {}^2V^{\text{E}} + (1-w)\frac{\text{d}[{}^2V^E]}{\text{d}w} \quad (4)$$

In the narrow range of w considered here (cf. Fig. 1), $V^{\text{E}}(S)$ was found to be constant, and thus equal to the infinite dilute value generally reported in the literature. We analyzed binary solutions of the smaller alcohols (1-octanol and shorter) and found good accordance with previously published values for $V^{\text{E}}(S)$ [23,24]. For longer alcohols, which are only sparsely soluble, and more demanding to analyze, we used literature values of $V^{\text{E}}(S)$ in the data treatment [23,24]. Since w is low enough for $V^{\text{E}}(S)$ to be independent of the composition, the excess partial specific volume of water in the mixtures is undetectably small, and ${}^2V^{\text{E}}$ can be approximated

$$ {}^2V^E \cong wV^{\text{E}}(S) \quad (5)$$

As discussed in detail elsewhere [9], the volumetric effects of membrane–solute interactions are reflected in the

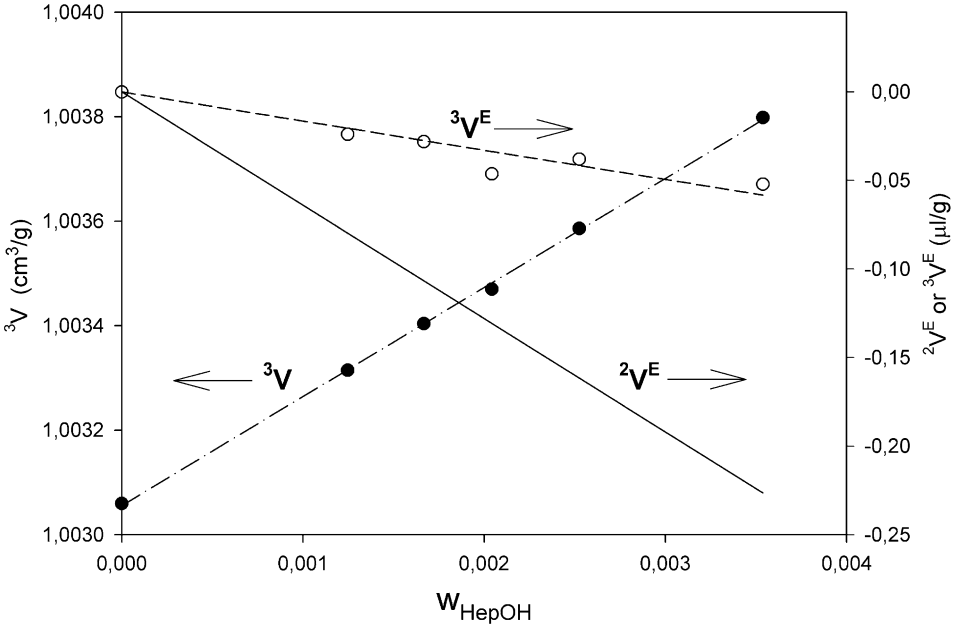

Fig. 1. Raw data from the densitometric measurements showing the specific volume of (DMPC-water-1-heptanol) suspensions, ${}^3V$, as a function of the alcohol weight fraction, $w_{1\text{HpOH}}$. Data points for ${}^3V$ are shown with filled symbols and the best linear fit with the dot-and-dash line. The excess volume functions ${}^3V^{\text{E}}$ (open symbols, dashed line) and ${}^2V^{\text{E}}$ (solid line) were calculated according to respectively Eq. (1) and Eq. (5), and also included in the figure (right-hand ordinate). The concentration on the abscissa refers to the total alcohol content. The concentration of alcohol in the membrane may be estimated by Eq. (7) and data in Table 1. This analysis showed that the alcohol concentration in the membrane covers the range 0.1–0.3 molecule of alcohol per molecule of DMPC for the five (non-zero) values of $w_{1\text{HpOH}}$ showed here.

difference between the excess volumes defined in respectively Eqs. (1) and (5). For the current type of data, the volume change, $\Delta V_m(\text{aq}\rightarrow\text{mem})$ of transferring one mole of solute from the aqueous bulk to a membrane partitioned state may be written

$$\Delta V_m(\text{aq}\rightarrow\text{mem}) = \left(\frac{d[^3V^E]}{dw} - V^E(S)\right) M_S x_S^{mem} \quad (6)$$

where M_S is the molecular weight of the solute and x_S^{mem} is the fraction of solute molecules which is partitioned into the membrane. The latter quantity can be expressed as [9]

$$x_S^{mem} = n_S^{mem}/n_S^{total} = \frac{K_P r_m}{1 + K_P r_m} \quad (7)$$

In Eq. (7), the mole ratio is expressed by the membrane–water partitioning coefficient, K_P (in molal units), and the lipid/water mass ratio in the sample, r_m.

To enable comparisons with solutes which are insoluble in water, we also calculated the molar volume change, $\Delta V_m(\text{pure}\rightarrow\text{mem})$, for the transfer from the pure organic liquid to the membrane partitioned state. Assuming again that the excess specific volume of the solute in aqueous solution is independent of w over the investigated range, the volume change of transferring solute from the neat organic liquid to aqueous solution is $M_S\ V^E(S)$. Hence, volume changes associated with the thermodynamic cycle of solute in the three states (pure, aqueous and membrane partitioned) can be written

$$\Delta V_m(\text{pure}\rightarrow\text{mem}) = \Delta V_m(\text{aq}\rightarrow\text{mem}) + M_S V^E(S) \quad (8)$$

3.2. Water insoluble solutes

For alkanes and higher alcohols which have negligible miscibility with water, the data analysis is simpler. As all the added solute is accumulated in the membranes while the aqueous bulk remains unchanged, it follows that the measured changes in volume can be assigned directly to the membrane phase and that $\Delta V_m(\text{pure}\rightarrow\text{mem})$ may be written

$$\Delta V_m(\text{pure}\rightarrow\text{mem}) = \frac{d[^3V^E]}{dw} M_S \quad (9)$$

It was found that data for 1-heptanol should be analyzed according to Eq. (8). For 1-octanol, which has $K_P \sim 480$ [3,20], insertion into Eq. (7) shows that $x_S^{mem} \sim 0.96$ (96% of the added alcohol is membrane bound) and volume changes calculated by either Eqs. (8) or (9) are practically equal. Obviously, this picture also prevails for still larger alcohols for which $x_S^{mem} \sim 1$. For the alkanes the water solubility is too sparse to measure $V^E(S)$ and the data for the three component systems can only be analyzed with respect to Eq. (9).

4. Results

Fig. 1 shows an example of volume data for the partitioning of 1-heptanol into multilamellar DMPC. The specific volume, ^3V, of six separate mixtures with different concentrations of alcohol was measured according to method 1 and plotted against the weight fraction of heptanol, $w_{1\text{HpOH}}$ (filled symbols, left hand ordinate). The dot-and-dash line is a linear fit to these raw data. The course of the ^3V function is dominated by the differences in volume of the pure components (the specific volume of the pure alcohol is 20% larger than that of water; hence the positive slope in Fig. 1) and volume changes associated with molecular interactions are therefore not readily seen in the ^3V function. To highlight effects of interactions, we calculated $^3V^E$ (Eq. (1)) and plotted it in the figure (open symbols, right-hand ordinate). Also included in the figure is $^2V^E$ (solid line, right-hand ordinate) calculated by Eq. (5) using $V^E(S) = -64$ μl/g [8,24]. It appears that $^2V^E$ and $^3V^E$ are negative. This shows that both the binary and tertiary mixtures have a smaller volume than their constituents in the standard state. More importantly, the fact that the excess volume in the three-component system (open circles) is larger (less negative) than the excess volume in the binary solutions (solid line) illustrates that the change in volume of transferring heptanol from water to a membrane partitioned state is positive (cf. Eq. (6)). We note that to within the experimental scatter (~0.005 μl/g) $^3V^E$ is proportional to $w_{1\text{-HpOH}}$. Hence, $\Delta V_m(\text{aq}\rightarrow\text{mem})$ is independent of the concentration of the alcohol (Eq. (6)). The slope of the (dashed) line fitted to the $^3V^E$ data is -15 ± 3 (SE) μl/g. Based on this uncertainty $\Delta V_m(\text{aq}\rightarrow\text{mem})$ was calculated (Eq. (6)) to 6.6 ± 1.0 cm^3/mol.

Data from the other nine alcohols were treated as described above for heptanol and the resulting values of $\Delta V_m(\text{aq}\rightarrow\text{mem})$ and $\Delta V_m(\text{pure}\rightarrow\text{mem})$ are shown in panel A of Fig. 2. Panel B of this figure shows the $\Delta V_m(\text{pure}\rightarrow\text{mem})$ values for the four alkanes calculated according to Eq. (9). The alkane samples were prepared by method 2. To check the conformity of the two methods, however, *c*-hexane and *n*-octane were also analyzed by method 1. No systematic differences between the two preparation methods were observed. Some of the alcohols, including 1-hexanol and 1-dodecanol, were analyzed using both extruded (unilamellar) and multilamellar DMPC. We did not find any systematic difference between the two types of samples. The data in Fig. 2 shows that $\Delta V_m(\text{pure}\rightarrow\text{mem})$ is consistently lower than $\Delta V_m(\text{aq}\rightarrow\text{mem})$. This reflects the negative sign of $V^E(S)$ (see Table 1 and Eq. (8)). The most important information in Fig. 2 is that the smaller alcohols transfer into the membrane with a volume increase while larger alcohols and alkanes, have negative ΔV_m values. It also appears that the expansion induced by alcohols is maximal for chain lengths (~6 carbon atoms) which are about half of that of the lipid fatty acids (14 carbon atoms). In other words, a mismatch between the alcohol and the lipid brings about a loosely packed complex while the alkanes and alcohols with approximately matching chain lengths enhance molecular packing. For the alkanes (panel B), it appears that the packing is better the smaller the solute. The volumetric data for membrane partitioning along with the key information used to calculate the membrane volumes are compiled in Table 1.

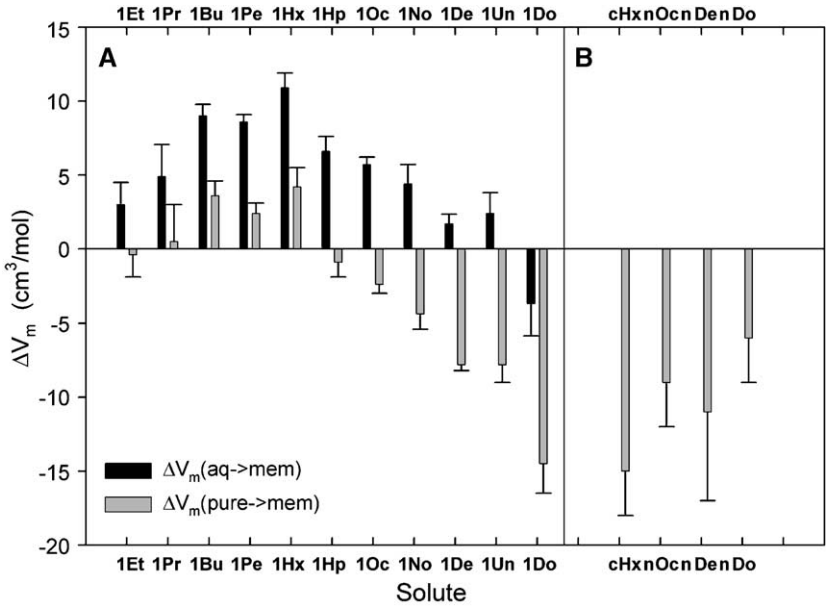

Fig. 2. Molar volume changes for the transfer of solutes from aqueous solution to the membrane partitioned state (ΔV_m(aq→mem), filled bars) and the transfer from the pure liquid state to the membrane partitioned state (ΔV_m(pure→mem), grey bars). The lipid membrane was DMPC in all cases and the experimental temperature was 30 °C. In addition to the 14 solutes investigated in this work, the figure includes data for ethanol [9]. Error bars reflect the repeatability of the current density measurements (see main text for details). For the smaller alcohols (hexanol and shorter) an additional uncertainty arise from the value of the partitioning coefficient K_p, introduced in Eq. (7). Rather deviant values of K_p have been published and the data in this figure is based on [1,3,20]. For the larger alcohols and the alkanes, K_p values have little or no influence on the results in the figure.

The apparent specific volume of lipid, V_{lip}^{App} was calculated from the measurements of V_{stock} (i.e. measurement of lipid suspensions without added solute)

$$V_{lip}^{app} = \frac{V_{stock} - (1 - w_{lipid}) V_{H2O}^{*}}{w_{lipid}} \quad (10)$$

where w_{lipid} is the weight fraction of lipid in the stock solution. We found that $V_{lip}^{App} = 976 \pm 3$ μl/g in good accordance with previously published values [9,25].

The DSC measurements were conducted to validate that membrane complexes of the larger solutes were in the fluid phase at 30 °C. For the smaller solutes, a comprehensive literature has established that the investigated systems are indeed in the fluid lamellar phase [3,14,19–21,26]. For the larger solutes tested by DSC here, we only found limitations for 1-dodecanol. This solute could only be studied at rather low concentrations (alcohol : lipid mole ratio less that 0.2) before T_m approached 30 °C. In this case the condensation of lipid into a gel phase generates a large negative volume change which makes interpretations along the lines described above impossible. From Fig. 2A it appears that it would be interesting to investigate still longer 1-alkanols to establish whether the decreasing tendency in ΔV_m tapers off at the same level as the alkanes. Attempts to do so, however, remained unsuccessful due to the formation of gel-phase at 30 °C and a strong increase in experimental scatter (possibly due to micro-bubbles in the densitometer) at higher experimental temperatures. The DSC data on the alkane samples showed decreasing T_m with solute concentration for octane and decane. For dodecane, T_m increased to about 28 °C for the highest concentrations studied here. This behavior is also in accord with previous DSC investigations [26]. In analogy to the alcohols, this tendency prevented volumetric investigations of higher alkanes at 30 °C.

The DSC traces did not show the multiple transitions characteristic for samples in which a part of the alkane is "frozen out" to a separate phase in the center of the membrane [26]. This may rely on the concentrations applied in the current work. Thus, for decane the gel-phase solubility in DMPC corresponds to a mole fraction of ~0.35 [26], i.e. higher than the concentrations used here, and consequently cooling to 10 °C in the calorimeter will not bring about the separation of an alkane phase in the membrane.

5. Discussion

Volumetric information has proven to be an effective tool in molecular descriptions of lipid membranes. For pure (one component) lipid bilayers, this has been developed to a sophisticated level [27,28] where combined experimental and theoretical efforts are used to quantify the volumes of moieties of the lipid molecule. Such "sub-volumes" are fundamental parameters for further modeling and useful in attempts to rationalize the molecular origin of experimental observations [27,28]. For binary (phospholipid + solute) systems, the use of volumetric information also appears to be important, but the available data – both experimental and computational – is too sparse to support even qualitative discussions of the relationships between the chemical structure of the solute and its packing properties (or excess volume) in the membrane. In fact, a number of reports on both alkanes and amphiphilic compounds in membranes have provided somewhat conflicting results [2,6–15,29].

Table 1
Volumetric data for the partitioning of alcohols and alkanes into multilamellar DMPC membranes at 30 °C

Solute	K_p	V^*_S	$V^E(S)$	ΔV_m(aq→mem)	ΔV_m(pure→mem)
		$cm^3\ g^{-1}$	$\mu l\ g^{-1}$	$cm^3\ mol^{-1}$	$cm^3\ mol^{-1}$
1-PrOH	1.3	1.255	−73	4.9±2.2	0.5±2.5
1-BuOH	3.3	1.247	−72	9.0±0.8	3.6±1.0
1-PeOH	12	1.240	−70	8.6±0.5	2.4±0.7
1-HxOH	26	1.233	−66	10.9±1.0	4.2±1.3
1-HpOH	135	1.227	−64	6.6±1.0	−0.8±1.0
1-OcOH	480	1.221	−62	5.7±0.5	−2.4±0.6
1-NoOH	–	1.218	−61	4.4±1.3	−4.4±1.0
1-DeOH	–	1.215	−60	1.7±0.6	−7.8±0.4
1-UnOH	–	1.212	−59	2.4±1.4	−7.8±1.2
1-DoOH	–	1.210	−58	−3.7±2.2	−14.5±2.0
c-hexane	–	1.300	–	–	−15±3
n-octane	–	1.440	–	–	−9±3
n-decane	–	1.385	–	–	−11±6
n-dodecane	–	1.348	–	–	−6±3

The two last columns lists the molar volume change, ΔV_m, associated with the transfer of a solute to the membrane from respectively dilute aqueous solution and the pure liquid state. The former of these functions could not be measured for the alkanes due to their low solubility in water. The table also lists the information used to calculate ΔV_m. The partitioning coefficients, K_p, are "best estimates" based on results in [1,3,20] and the requirement that a plot of $\ln(K_p)$ vs. the number of carbon atoms in the 1-alkanol chain should be a smooth curve. Partitioning coefficients for the more hydrophobic solutes were not used in the data treatment (see text) and are therefore not listed. The specific volume of the pure organic liquids, V^*_S, was measured in this work. The excess partial specific volume of the solute, $V^E(S)$, in dilute binary solution was measured for the smaller alkanols (1-octanol and shorter). The results were in good accordance with earlier reports [23,24]. Hence, this literature data [23,24] was used for $V^E(S)$ for the larger alcohols in the table.

In the current work, we have used density measurements to evaluate the packing of solutes in fluid lipid bilayers. This approach is based on the assumption that the foreign molecule does not bring about gross structural changes in the membrane, and thus that the measured density change signifies packing of solute in intact bilayers. To ensure this, we investigated only dilute samples, in which the lipid : solute mole ratio was varied within in the range of 3–10. At these concentrations the main structure of the membrane has previously been shown to be conserved (see [26,30–35] and references therein). Some general relationships of packing and solute structure may be derived from the results. Hence, 1-alkanols with chain lengths of about 4–7 carbon atoms generate a moderate loosening when entering the membrane (Fig. 2A). Shorter alkanols have a limited effect on the volume, while partitioning of long-chain alcohols show a clear negative ΔV_m(pure→mem) and thus promote molecular packing. Alkanes also promote packing; more so the smaller the solute.

The values found here are in accordance of those for 1-heptanol and 1-octanol reported by Kita and Miller [8]. Also, ΔV_m(aq→mem) for small alcohols (1-propanol and 1-butanol) are in perfect accordance with those previously reported by us, using a different methodology [9]. For 1-pentanol and 1-hexanol, however, our previous ΔV_m-values are larger than those found here. Closer inspection shows good accordance of $^3V^E$ in the two works, but $^2V^E$ in the original work is more negative that the current data and results from the literature on binary aqueous alcohols [24]. The difference is not a result of experimental scatter (the $^2V^E$ function was reproducible in both works), but may be due to the fact that the earlier work used a phosphate buffer with NaCl as solvent (while the solvent here is pure water). It may also reflect that the automated equipment used in the original paper is inadequate for analysis of larger alcohols with low solubility in water. At any rate, this discrepancy is only pertinent to ΔV_m(aq→mem) since ΔV_m(pure→mem) is independent of the volume of the solute in aqueous solution.

To interpret the results we briefly review some relevant structural information for lipid bilayer membranes and their complexes with alcohols and alkanes. It has been established that alcohols are predominantly anchored with the hydroxyl group at the polar interface and the acyl chain intercalated between the acyl chains of the lipids [30–32,36–38]. Alkanes, on the other hand accumulate in the interior of the membrane near the methyl groups of the fatty acids ([26,31,32,39,40]. Although different conclusions have been made, strong evidence supports the suggestion that alkanes are primarily oriented along the normal to the plane of the membrane, particularly so for chain lengths exceeding ~10 carbon atoms [26,31,32]. Structural investigations of pure lipid membranes have shown that electron density varies strongly with the depth [41]. Hence, a maximum is observed around the glycerol backbone while a pronounced dip is found in the membrane core near the fatty acid methyl groups. This depth-dependence of the packing properties was further investigated in a computer simulation study by Marrink et al. [42], who found that the occurrence of accessible free volume or "voids" in the membrane was low (i.e. tight packing) in the region around the first few segments of the acyl chain. Conversely, free volume was prevalent in the core of the membrane.

Based on the reported ΔV_m(pure→mem) values and this structural information, we suggest a simple packing model based on two dominant effects. These are (i) intercalation of solute in the outer region of the membrane and (ii) entry of solute into the core of the membrane. The two effects are suggested to contribute to ΔV_m(pure→mem) with opposite signs. Hence, (i) will perturb the tight interfacial packing of the lipids. This will lead to less dense packing particularly at positions deep enough *not* to be reached by the solute. In other words, since the solute is lodged with the hydroxyl group near the water–membrane interface, the mismatch between the acyl chains of respectively solute and lipid will generate additional free volume in the core of the complex. Conversely, (ii) will generate a negative contribution to ΔV_m(pure→mem) since the solute (or part of it) will dissolve in a loosely packed region (the membrane core). It will tend to occupy some of the free volume, which is abundant here, and hence promote a denser packing.

The data in Fig. 2A may be rationalized along these lines. Thus, the maximum in ΔV_m(pure→mem) for alcohol chain lengths (C5–C8) around half of the lipid fatty acids (C14) signifies the culmination of the "mismatch effect" in (i). For smaller solutes, the penetration is rather shallow and the effect of (i) concomitantly small. For longer alkanols, ΔV_m(pure→

mem) decreases with the chain length since the negative contribution from (ii) becomes increasingly important (see below). As a result of the looser packing in the membrane core, (i) is expected to increase in the occurrence of gauche conformers in the lipid acyl chains in this zone. Macroscopically, this would result in thinning of the membrane, and several studies have confirmed this consequence of amphiphile partitioning. In particular, Uhrikova et al. [43] reported that a homologous series of carbamic acid esters with chain lengths ranging from 3 to 12 carbon atoms resulted in membrane thinning with a maximum effect for solute chain lengths around half the lipid acyl chain. In fact, the dependence of the membrane thickness on solute chain length, found in the work of Uhrikova et al., corresponds qualitatively to the course of ΔV_m(pure→mem) in Fig. 2A, and the interpretation proposed by Uhrikova et al. is comparable to the picture discussed here. Another analogy to the course in Fig 2A comes from studies of alcohol effects on ion-permeability of PC-vesicles [44]. This work reported the permeability of vesicles doped by a homologous series of 1-alkanols, and found that mid-sized alcohols (C5–C7) were particularly strong in breaking down the membrane permeation barrier. This is in accord with the current results since a thinner and looser membrane is expected to produce a weaker barrier.

The volume changes illustrated in Fig. 2A gradually decrease for alcohols chain lengths exceeding 6 carbon atoms. We suggest that this reflects the importance of (ii) and that the magnitude of this effect may reach about −10 to −15 cm^3/mol. As discussed above, experimental limitations prevent us from investigating larger alcohols (and thus establish whether the effect remains at this level). However, the reasonable match in chain length of dodecanol and the lipid acyl chains as well as the observation that ΔV_m(pure→mem) for dodecanol is similar to that of the alkanes suggest that −10 to −15 cm^3/mol may approach the maximal effect of (ii). In addition to the negative volume changes for the alkanes, we note that the change tends to become less negative as the alkane chain length increases. This may also be related to the balance of (i) and (ii). Thus larger alkanes are suggested to exhibit a time averaged position normal to the membrane surface (i.e. along the lipid acyl chains) while shorter alkanes accumulate in the membrane core near the methyl groups of the lipids [26,31,32]. It follows that long chains are likely to span a large part of the hydrophobic region and thus penetrate into the tightly packed zone. Indeed, penetration into this zone and the hydrated membrane interface has been suggested to underlie the limited solubility of long-chain alkanes in lipid bilayers [26]. Disruption of the tight packing here will bring about a positive contribution to ΔV_m in accordance with the trend observed for longer alkanes in Fig. 2. Short-chained alkanes, on the other hand, primarily accumulate in the more disordered core resulting in more negative ΔV_m-values.

Uhrikova et al. [34,35] have recently conducted a thorough study on the effect of *n*-decane on the thickness of POPC membranes. They concluded that for the concentrations used in the current study, decane had practically no effect on the thickness. If these results on an unsaturated lipid are applicable to the lipid used here (DMPC), the large negative ΔV_m for decane (Fig. 2B) suggests that this solute enhances lateral packing. This effect may be even more pronounced for the smaller alkanes, which tend to increase membrane thickness although they have the most negative ΔV_m. Further insight into the extent of this effect appears central for the understanding of solute induced effects (e.g. anesthesia) on biological membranes (cf. [45,46]).

In conclusion we have established some relationships between the chemical structure of a solute and its packing properties in fluid DMPC membranes. We have also suggested a molecular interpretation which rests on the distribution of free (interstitial) volume in the membrane and the structure of membrane–solute complexes. The interpretation is in accord with the observation that the methylene segments near the end of the lipid acyl chain increase their motional freedom upon insertion of mismatched (C8) alcohols [30,36,38] or surfactants. [47] It is also corroborated by the report by Coster and Laver [17] which showed that the insertion of a mismatched alcohol facilitates the partitioning of alkanes into the PC membrane. In analogy to the current discussion, Coster and Laver suggested that this was due to an alcohol induced loosening of the membrane core. Further analysis of this packing model including experimental investigations of membrane–fatty acid interactions and computer simulations of the distribution of free volume in membrane–alcohol complexes is currently pursued.

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