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Molecular packing in 1-hexanol–DMPC bilayers studied by molecular dynamics simulation

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Abstract

The structure and molecular packing density of a "mismatched" solute, 1-hexanol, in lipid membranes of dimyristoyl phosphatidylcholine (DMPC) was studied by molecular dynamics simulations. We found that the average location and orientation of the hexanol molecules matched earlier experimental data on comparable systems. The local density or molecular packing in DMPC–hexanol was elucidated through the average Voronoi volumes of all heavy (non-hydrogen) atoms. Analogous analysis was conducted on trajectories from simulations of pure 1-hexanol and pure (hydrated) DMPC bilayers. The results suggested a positive volume change, ΔV_m, of 4 cm^3 mol^{-1} hexanol partitioned at 310 K in good accordance with experimental values. Analysis of the apparent volumes of each component in the pure and mixed states further showed that ΔV_m reflects a balance between a substantial increase in the packing density of the alcohol upon partitioning and an even stronger loosening in the packing of the lipid. Furthermore, analysis of Voronoi volumes along the membrane normal identifies a distinctive depth dependence of the changes in molecular packing. The outer (interfacial) part of the lipid acyl chains (up to C8) is stretched by about 4%. Concomitantly, the average lateral area per chain decreases and these two effects compensate so that the overall packing density in the outer region, where the hexanol molecules are located, remains practically constant. The core of the bilayer (C9–C13) is slightly thinned. The average lateral area per chain in this region expands, resulting in a looser packing density. The net effect in the core is a 2–3% decrease in density corresponding to a total volume increase of ~14 cm^3 mol^{-1} hexanol partitioned.

Keywords: Volume change; Packing density; Lipid membrane; Mismatch; Membrane–solute interaction

1. Introduction

Lipid bilayer membranes are highly anisotropic. As a result, introduction of other molecules ("solutes") into the lipid matrix brings about changes in the structure and properties of the membrane, which are more pronounced than those typically seen for solute effects in an isotropic solvent. The changes in dimensions of the membrane following the addition of solute, for example, are complex and often involve decoupling of the expansion in the normal and lateral directions [1]. In many cases, changes along the two directions carry opposite signs [2–7]. Understanding these solute induced modulations of lipid membranes is fundamental to the description of numerous biological processes. Thus, the biological membrane interacts with an array of solutes including ions, osmolytes, metabolites and cholesterol, and these interactions change the structure, dynamics and mechanical properties of the lipid matrix. This, in turn, may couple to protein function and hence the activity of the biomembrane. Possible mechanisms underlying this coupling may rely on matching of the thickness of the membrane and the hydrophobic surface of transmembrane proteins [8], lateral organization and the formation of rafts in the lipid matrix [9], or the lateral pressure profile, which generates pronounced effects for an extremely thin film like a fluid bilayer [10,11]. A pronounced sensitivity to solute induced effects is common to all of these putative mechanisms, and it is therefore of interest to elucidate general relationships between the chemical structure of a solute and its effect on lipid membrane properties. One particularly elegant example of such interrelationships comes

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from studies of sterols. Thus, the smooth and rigid structure of the steroid group of cholesterol in mammalian cell membranes promotes the formation of the so-called liquid ordered (lo) phase. Its biosynthetic precursor, lanosterol, has three protruding methyl groups which make the steroid surface somewhat more rugged. This seemingly minor structural difference, however, is enough to render lanosterol unable to introduce the degree of lipid ordering which is required for the lo phase [12,13].

In the current work, we investigate the molecular packing of the solute, 1-hexanol, in a DMPC bilayer. The choice of this system was motivated by a recent report in which the volume change for solute partitioning into DMPC was investigated experimentally for a homologous series of alcohols (along with some alkanes) [14]. This work showed that 1-alkanols with an intermediate chain length (e.g. butanol, pentanol and hexanol) partition into DMPC with a positive excess volume. This implies that the volume of the mixed membrane-alcohol system is larger than the sum of the constituents (respectively liposomes in dilute aqueous dispersion and pure liquid alcohol). The positive excess volume shows that the amount of free or "interstitial" volume increases as a result of the partitioning process—i.e. that mixed membrane–alcohol system is less effectively packed. It was found that this effect culminated for alcohols with chain lengths about half the lipid acyl chain. Still longer alkanols (C8–C12) in DMPC membranes were characterized by pronounced negative excess volumes. For 1-dodecanol, with a chain length almost matching that of the lipid, the volume change was about −14 cm^3 mol^{-1} alcohol partitioned. This suggested that dodecanol packed efficiently into the membrane by utilizing some of the interstitial volume available in the pure bilayer. These volumetric results pointed towards some general relationships between the size and location of the solute on one hand and its effect on the structure and molecular packing of the membrane complex on the other. Hence, it was suggested that solutes located around the first few carbon segments of the fatty acids reduce the over all molecular packing of the membrane, while solutes near the terminal methyl group tend to promote packing efficiency [14]. This suggestion is in accord with some previous work [1,7], and may rely at least in part on the balance between tight average packing of the first few methylene segments in pure membranes and the higher abundance of free volume near the membrane core [15,16].

To investigate the effects of solutes on membrane packing further, we have conducted a molecular dynamics simulation study on 1-hexanol–DMPC mixtures. This system showed the largest excess volume in the experimental work [14] and is therefore expected to reveal the characteristic properties most conspicuously. Hydrophobic alcohols in lipid bilayers have not previously been investigated by MD simulations, which have addressed only smaller alkanols [3,17,18].

2. Methods

2.1. Simulations

Molecular dynamics simulations of a pure DMPC membrane, a DMPC membrane embedded with 1-hexanol (DMPC/*n*-hexanol) and bulk 1-hexanol were performed using the program NAMD [19] with the CHARMM27 all hydrogen parameter set [20]. The distribution of charges in the DMPC head group were modified according to the methodology described by Sonne at al. [21]. The TIP3 water model was used.

The initial structure was taken from a membrane consisting of 128 DMPC molecules and water previously equilibrated at 330 K and cooled to the current temperature (see below). To ensure hydration of the enlarged area in the simulations of mixed DMPC–hexanol, additional water was added, to a total of 4952 molecules (~39 water per lipid). This system was simulated both alone and following the insertion of a 5×5 grid of 1-hexanol molecules in each monolayer (i.e. a total of 50 alcohol molecules corresponding to a mole fraction $x_{hex}=0.28$ in the membrane. This is similar to the higher of the concentrations used in our experimental study [14]). In the initial position, the 1-hexanol molecules were aligned with the membrane normal and the oxygen located in the same plane as the DMPC carboxyl. We also simulated pure liquid 1-hexanol by randomly placing 512 molecules in a cube with an edge length of 47 Å, which corresponds to the macroscopic density. To avoid overlapping of atoms, the energy was minimized for 2000 steps.

The simulation was performed using a Verlet velocity integrator [22] with a timestep of 1 fs in a Cartesian box with periodic boundary conditions, a constant ambient pressure at 1 atm and a constant temperature at 310 K. The pressure was held constant using a Langevin piston method [23] with a damping coefficient of 5 ps^{-1}, a piston period of 200 fs and a piston decay of 500 fs. The fluctuations of the cell were done anisotropically in the simulations of the membranes and isotropically in the simulations of bulk 1-hexanol. The long range electrostatic forces were calculated using the Particle Mesh Ewald method [24]. The grid spacing was approximately 1 Å and a fourth order spline was used for the interpolation. The calculation was performed for every fourth femtosecond. The van der Waals interactions were cut off at 12 Å using a switching function starting at 10 Å. Pair lists within a distance of 14 Å were used. The total simulation times of the membranes and bulk hexanol were respectively 90 ns and 10 ns.

Voronoi volumes of all the heavy atoms were calculated following the removal of hydrogen atoms and used to elucidate the molecular packing [25,26]. The Voronoi polyhedron for a given atom encloses all the points in space that have the atom as its nearest neighbor and its volume is denoted the Voronoi volume. We analyzed approximately 30 configurations separated by 1 ns from each membrane simulation. The separation between each configuration of bulk hexanol was 0.1 ns.

3. Results

3.1. Lateral areas

The time course of the lateral surface area of the membranes throughout the simulations is illustrated in Fig. 1. Since the initial set-up was a membrane at a somewhat higher temperature (see above), the area decreased with time in the early parts of the simulations. Therefore, we limited the statistical analysis to the 35–85 ns time range.

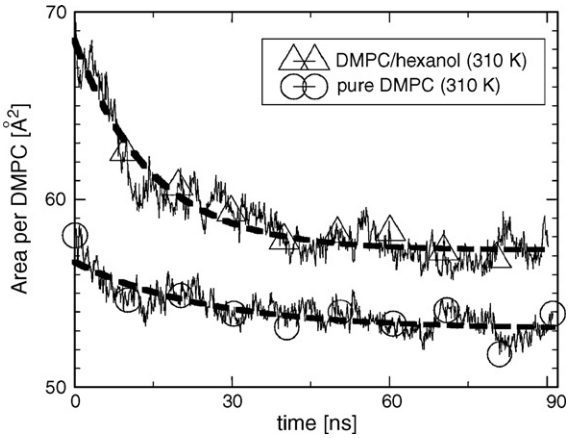

Fig. 1. Area per DMPC molecule as a function of the simulation time. The lower curve (circles) represents pure DMPC membranes, while the upper (triangles) is DMPC–hexanol with an alcohol mole fraction of 0.28 (see text for details).

The higher surface area in the mixed DMPC–hexanol system (Fig. 1) reflects the lateral expansion associated with the insertion of the alcohol molecules. To quantify this effect, we calculated the average lateral area per molecule A_{mol} (hexanol or lipid) and the average lateral area per acyl chain A_{chain} (i.e. myristic acid or hexanol) and listed the values in Table 1 together with the equilibrium values of the areas, A_{DMPC}, from Fig. 1.

It appears from Table 1 that the average lateral area per chain is ~10% smaller in the alcohol doped systems. In other words, the hexanol chain contributes less to the lateral area than the fatty acids of DMPC. The contribution of hexanol towards the total lateral area may be quantified by the apparent lateral area of the alcohol, ${}^{\mathrm{app}}A_{\mathrm{hex}}$.

$$ {}^{app}A_{\mathrm{hex}} = \frac{{}^{mix}A_{\mathrm{chain}} - (1 - {}^{chain}x_{\mathrm{hex}})\,{}^{pure}A_{\mathrm{chain}}}{{}^{chain}x_{\mathrm{hex}}} \quad (1) $$

where ${}^{\mathrm{chain}}x_{\mathrm{hex}}$ is the mole fraction of hexanol chains,[2] and ${}^{\mathrm{pure}}A_{\mathrm{chain}}$ and ${}^{\mathrm{mix}}A_{\mathrm{chain}}$ are respectively the chain lateral area of pure DMPC and the DMPC–hexanol mixture taken from Table 1. Insertion into Eq. (1) shows that ${}^{\mathrm{app}}A_{\mathrm{hex}}$ is about 11 Å^2. It follows that the addition of one hexanol chain brings about only ~40% the lateral expansion as one lipid chain in pure DMPC.

The value of ${}^{\mathrm{app}}A_{\mathrm{hex}}$ compares favorably with experimental data on comparable systems. Thus, an apparent area of 17–18 Å^2 can be derived for 1-octanol in DMPC on the basis of the NMR measurements by Pope et al. [27]. Also, we estimated an ${}^{\mathrm{app}}A$ value for 1-butanol in SOPC of about 12 Å^2 using the micropipette aspiration data by Ly and Longo [6]. The average area, A_{DMPC}, from the simulations of pure lipid is ~10% smaller than experimentally determined lateral areas [28]. A part of this discrepancy may be related to the finite size of the simulated membranes [29].

[2] Since there are 128 lipids with two acyl chains and 50 hexanol, ${}^{\mathrm{chain}}x_{\mathrm{hex}}$ is 50/(50+2(128))=0.16.

Table 1
Average lateral areas of the equilibrated systems

	A_{DMPC} (Å^2)	A_{mol} (Å^2)	A_{chain} (Å^2)
DMPC	53.1	53.1	26.5
DMPC–hexanol	57.3	41.2	24.0

The total lateral area was normalized by respectively the number of DMPC molecules (A_{DMPC}), the total number of molecules (A_{mol}) and the total number of acyl chains (fatty acid or hexanol) (A_{chain}).

3.2. Depth dependent structure

The distribution of water, hexanol and selected lipid moieties along the z-axis (i.e. normal to the membrane plane) is illustrated in Fig. 2. The upper and lower panels illustrate respectively pure DMPC and DMPC–hexanol. Although this presentation provides only a coarse picture of the structure, it illustrates that the hexanol molecules accumulate in the outer part of the hydrophobic region, slightly deeper than the membrane head group. Very little hexanol was found in the membrane core near the lipid methyl groups (near $z=0$ in Fig. 2). This picture is in accordance with experimental investigations of medium-length alcohols in lipid bilayers [27,30–35]. These works collectively showed that alcohols intercalated into the bilayer with the –OH group around the polar head group of the

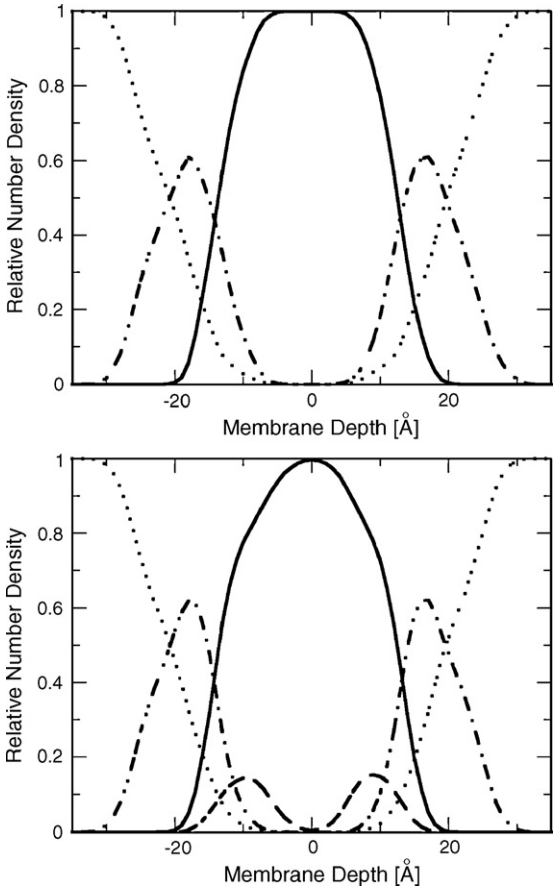

Fig. 2. Relative distribution of water (dotted), head group (dot-and-dash), acyl chain (solid) and hexanol (dashed) as a function of the depth, z ($z=0$ corresponds to the geometric center). The upper panel is a pure DMPC, while the lower is DMPC–hexanol.

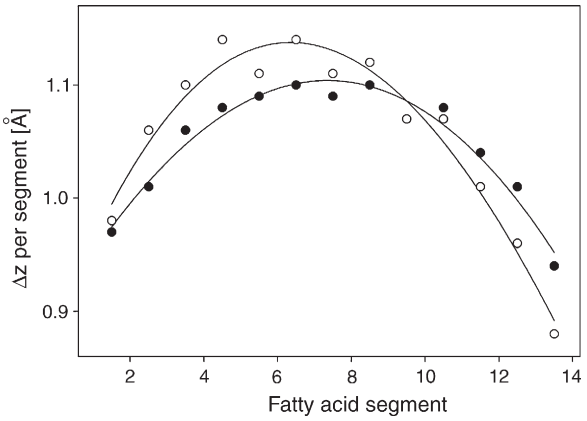

Fig. 3. Average length along the z-axis, Δz, of the methylene groups in the sn-2 fatty acid as a function of the depth in the membrane (the distance between, e.g., C4 and C5 is plotted at an abscissa value of 4.5). The filled symbols represent the pure DMPC membrane, while open symbols are DMPC–hexanol. It appears that hexanol elongates the outer part of the hydrophobic region in the normal direction. Conversely, the core region becomes thinner.

lipid. Detailed, tabulated information on the average z-position of all atoms in the simulations can be found in the supplementary material. A few examples of particular importance for the current discussion will be mentioned here. The average position (distance to geometrical center) of the oxygen in hexanol is 13.0±2.4 Å (std.). This value matches the position of the third carbon in the fatty acid chains. The methyl group of the hexanol molecules was found to be 6.8±2.3 Å (std.) from the membrane center, corresponding to a position between C8 and C9 in the fatty acid chain. It follows that the seven heavy atoms of hexanol span a distance corresponding to ~6.5 fatty acid segments. This is due to a larger average tilt of the shorter molecule and the less restricted movement of the alcohol methyl group. To further investigate the structure along the membrane normal, we calculated the average segment length (along the z-axis) for the fatty acids. It appeared (Fig. 3) that the average progression, Δz, per segment increased from ~0.97 Å at the interface to ~1.09 Å at the middle of the fatty acid of pure DMPC. Addition of hexanol increased these values by about 3–5% in the zone where hexanol partitions (C3–C9). At deeper positions, Δz is smaller for the mixed system. This implies that hexanol "stretches" the outer half of the hydrophobic zone, while the membrane core is condensed in the normal direction.

3.3. Voronoi volumes

The simulations of pure liquid hexanol, pure DMPC membranes and mixed hexanol–DMPC membranes were analyzed with respect to the Voronoi volume. Some key numbers are listed in Table 2 and detailed results listing the average volume of all (non-hydrogen) atoms of hexanol and DMPC can be found in the supplementary material.

Comparisons of experimental data and the Voronoi volumes of the pure components in Table 2 show deviations of respectively 2% and 4% for hexanol [36] and DMPC [37,38]. It appears that the Voronoi volume of a lipid molecule in the membrane expands by about 5 cm^3 mol^{-1} upon addition of 1-hexanol to a mole fraction of 0.28. Conversely, the volume of membrane partitioned hexanol is about 9 cm^3 mol^{-1} smaller than in the pure state. The total volume change of the system, ΔV_{mix}, associated with the transfer of one mole of alcohol from the pure to the membrane partitioned state may be written

Table 2
Voronoi volumes in cm^3 mol^{-1} for 1-hexanol and DMPC in their pure states and in mixtures

1-Hexanol		DMPC		ΔV_{mix}
Pure liquid	In membrane	Pure	W. hexanol	
130.1±0.04	120.8±0.14	632.7±0.17	637.9±0.18	+3.9

The volume change, ΔV_{mix}, calculated according to Eq. (2) signifies the total volume change following the transfer of 1 mol of alcohol from the pure state to a membrane partitioned state with a mole fraction of x_{hex}=0.28.

$$\Delta V_{mix} = \frac{N_{hex}({}^{vor}V^{mix}_{hex} - {}^{vor}V^{hex}_{pure}) + N_{DMPC}({}^{vor}V^{mix}_{DMPC} - {}^{vor}V^{pure}_{DMPC})}{N_{hex}} \quad (2)$$

where N identifies the number of molecules and ${}^{vor}V$ are the Voronoi volumes listed in Table 2. Subscripts indicate the component (DMPC or hexanol) and superscripts show the state (pure or mixed). The value of ΔV_{mix} calculated from Eq. (2) may be compared to transfer volumes measured experimentally. We have recently found ΔV_{mix}=4.2±1.3 cm^3 mol^{-1} for 1-hexanol in DMPC at 303 K [14] in good accordance with the data in Table 2.

The depth dependence of the Voronoi volumes is illustrated in Fig. 4. This figure shows that the average molecular packing of the outer lipid segments remains practically unchanged upon the addition of hexanol (filled and open symbols are superimposed). At positions deeper than ~C8, on the other hand, hexanol brings about a loosening of the lipid chains. Integration of the area between the two DMPC curves in Fig. 4 suggests that the volume in the membrane core is increased by 5.5–6.0 cm^3 mol^{-1} DMPC (or ~14 cm^3 mol^{-1} added hexanol). Owing to statistical

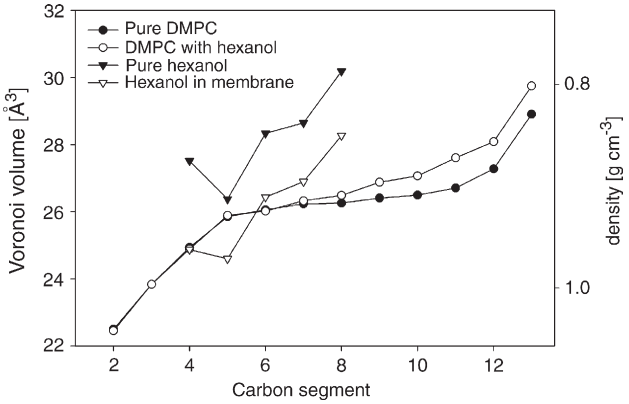

Fig. 4. Voronoi volumes of methylene segments of respectively hexanol and the sn-2 chain of DMPC. The abscissa is the carbon atom number of the lipid chain (C2 is the first methylene segment) and the left-hand ordinate gives the volumes in $Å^3$. The right-hand axis gives the results in density units. Hexanol is plotted with the first methylene segment at C4 in accordance with the average position of the alcohol.

Table 3
Average dimensions of the hydrophobic part of DMPC and DMPC–hexanol at 310 K

Compound	Position	Δz (Å)	^{Vor}V (Å^3)	A^* (Å^2)
DMPC (pure)	Outer	1.062	25.19	23.7
DMPC (mix)	Outer	1.103	25.20	22.8
Hexanol (pure)	–	–	28.22	–
Hexanol (mix)	–	1.049	26.22	25.0
DMPC (pure)	Core	1.030	27.23	26.5
DMPC (mix)	Core	1.026	27.92	27.2

The data for the lipid molecules was pooled and averaged in two groups corresponding to respectively the outer (C2–C8) and core (C9–C13) parts of the fatty acid chains. The three last columns list respectively the average progression in the direction of the z-axis ($\Delta z \pm 0.01$ Å), the average Voronoi volume ($^{Vor}V \pm 0.03$ Å^3) and the average lateral area ($A^* \pm 0.1$ Å^2). The latter quantity is calculated as $^{Vor}V/\Delta z$. All values are per methylene segment and the uncertainties listed above (in brackets) are standard errors of mean.

limitations, the results for hexanol in Fig. 4 are somewhat more scattered, but it appears that partitioning into the membrane lowers the volume of hexanol methylene segments by 7–11% throughout the length of the molecule. The Voronoi volume of the hydroxyl group (not shown) was found to decrease more (~17%) upon transfer to the membrane. Except for the last methylene segment, the packing density of the alcohol is rather similar to that of the adjacent part of the DMPC chains.

It appears from Figs. 3 and 4 that hexanol has qualitatively different effects on the structure of the outer (C2–C8) and core (C9–C13) parts of the fatty acid chains. To illustrate this, Table 3 compiles data on the average dimensions of these two regions. These results show that insertion of hexanol stretches the outer part of the fatty acids by about 4% while leaving the volume (and hence density) of this zone largely unchanged. For the inner (core) part of the fatty acids, the length along the z-axis shrinks slightly and the volume increases by a 2–3%. Also listed in Table 3 is the available lateral "segment area" defined as $A^* = {}^{vor}V/\Delta z$.

One important physical property often discussed on the basis of free volume considerations is the lateral diffusion coefficient. To investigate possible volume-diffusion correlations for this system, we determined the mean square displacement, $\langle r^2 \rangle$, of the phosphorous atom in DMPC and the first carbon in hexanol as a function of time and plotted the function $1/4\langle r^2 \rangle/t$ vs. the logarithm of time in Fig. 5. In a homogenous two-dimensional system, $1/4\langle r^2 \rangle/t = D$, where D is the diffusion coefficient [39]. Fig. 5 clearly shows that time scales in the order of a few dozen of ns are too short to get a constant value of $1/4\langle r^2 \rangle/t$ and hence a measure of D. This is expected since the mobility on short time scales will be influenced by the "ballistic" movement in regions where high free volume occurs momentarily [39]. This phenomena is also seen in experiments [40], and it has been suggested that it may require a movement of ~10 molecular diameters to establish macroscopic diffusion coefficients [39]. Nevertheless, it appears from Fig. 5 that D is approaching constancy for the longest simulation times. The value here corresponds to about $D_{DMPC} = 8 \times 10^{-8}$ cm^2/s in good accordance with experimental results for the movement of a phospholipid probe in DMPC at 34 °C (6.9×10^{-8} cm^2/s, [41]). Furthermore, extrapolation to ~100 ns (see curve on Fig. 5) suggests a D value very close to the value measured [41]. A similar analysis of the mixed membrane suggests that D for the lipid is almost twice the value in pure DMPC membrane (~12×10^{-8} cm^2/s), whereas the diffusion coefficient for the alcohol is about 14×10^{-8} cm^2/s.

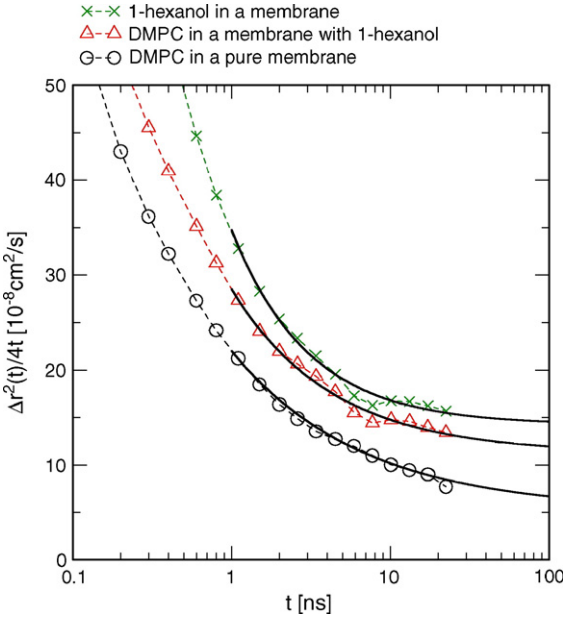

Fig. 5. A logarithmic plot of the time dependence of the mean square displacement divided by time, $\langle r^2 \rangle/4t$. At long time scales, this function is equal to the diffusion coefficient (see text). Circles, triangles and crosses represent respectively DMPC in pure membranes, DMPC in mixed DMPC–hexanol and hexanol in mixed membranes. The lines are power law fits.

4. Discussion

The structure of the hexanol–DMPC system found in this work conforms with experimental investigations of mid-sized alkanols partitioned into lipid bilayers. Thewalt et al. [31] concluded from deuterium NMR studies on DPPC that 1-decanol intercalated approximately parallel to the fatty acids covering the C3–C13 region. Similar results have been reported for related systems [27,30,32]. The location of hexanol found in this work (along the C3–C9 stretch of the fatty acids) was rather

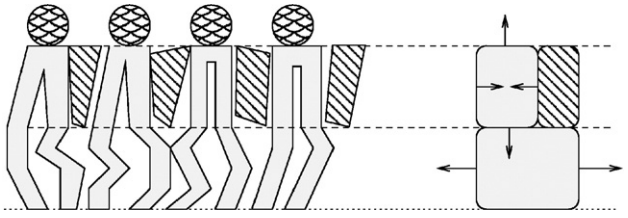

Fig. 6. Overview of the structural changes associated with the insertion of 1-hexanol into DMPC membranes. The illustration only shows one monolayer. The intercalated alcohol molecules (hatched) are located with the hydroxyl group near C2 of the fatty acid chains. The right-hand side of the figure illustrates the effect of the alcohol on the average dimensions of the lipid molecules. The two gray rectangles represent respectively the inner (C9–C13) and outer (C2–C8) parts of the hydrophobic region and the arrows indicate the changes in size of these zones resulting from the insertion of the solute.

stable. Thus, "out-of-plane" fluctuations assessed by the standard deviation on the average z-position was similar for adjacent segments of lipid and alcohol. Also, hexanol molecules placed close to the membrane core (in separate simulation trials) were found to travel to the equilibrium (interfacial) position at a time scale of ~10 ns and no solute molecules moved to the aqueous phase during the course of the simulations. The experimentally determined partitioning coefficient for 1-hexanol into saturated PC membranes is about 900 in mole fraction units [42,43]. For the current systems, this value translates into an equilibrium condition with 1 or 2 hexanol molecules in the aqueous phase. Equilibration of hexanol partitioning, however, is a very slow process [14,44] and certainly not expected within the present time scale.

The main scope of this work is to elucidate the packing properties of a mismatched solute molecule in a lipid bilayer. Earlier reports have suggested that solutes with hydrophobic chains of about half the length of the lipids strongly perturb the physical properties of a membrane [1,7,10,30,33–35,45]. We recently suggested that a mismatched solute such as hexanol, which is lodged with one end near the membrane–water interface leaves a particularly loosely packed zone at positions deep enough not to be directly effected by the solute [14]. It was proposed that this was the origin of the positive excess volume for hexanol–DMPC mixtures and, in turn, a possible mechanism underpinning the perturbing effects of mismatched solutes mentioned above. The current work provides the basis for a more detailed discussion of the packing of mismatched solutes. Thus, the less effective packing of the membrane core is directly quantified in Table 3. We found a 2.5% increase in the volume of the C9–C13 segments in the hexanol doped membranes. Closer inspection reveals that this expansion of the membrane core represents a balance between opposing lateral and normal contributions. The average length along the z-axis of this moiety (C9–C13) decreased (Table 3, Fig. 3), while the average available lateral area, A^*, expanded by ~3% (Table 3). For the upper part of the membrane chains (C2–C8), which are in direct contact with hexanol, the effect of the alcohol is less pronounced. Thus, the lipid Voronoi volume (per methylene group) remains practically unchanged (Fig. 4, Table 3). Furthermore, the average volume for C1–C4 of the alcohol molecule is similar to that of the adjacent lipid methylene groups (Fig. 4), and it follows that the overall density of this part of the membrane (C2–C8) is very similar in pure DMPC and in DMPC–hexanol. The constant net density, however, relies on compensating changes (~4%) in thickness (increasing) and area (decreasing) of this zone. An overview of these packing effects in hexanol–DMPC is illustrated in Fig. 6.

The results in Fig. 5 exemplify that, although the average changes in molecular packing are moderate, distinctive changes may occur in physical properties such as the lateral diffusion, which is increased by a factor of two upon the addition of hexanol. An earlier experimental study [46] also showed a change in D of about a factor of 2 when a solute (cholesterol) was added to a mole fraction of about 0.3. In this case, however, the diffusion was lower in the mixed system, and it was suggested that this relied on the ability of the sterole to promote the average molecular packing (i.e. to reduce free volume). These results emphasize the strong coupling between lipid packing and lateral diffusion.

The ability of a solute to affect the order of lipid chains has been highlighted in several discussions of solute–biomembrane interrelationships [47–52]. The current results (Table 1 and Fig. 4) together with experimental data [31,32,35] suggests that a 1-alkanol of about half the length of the fatty acid chains orders the adjacent lipid segments even though the inserted alcohol chain is fully flexible. Lipid segments at deeper positions experience a greater motional freedom following intercalation of the alcohol [27,30]. Insertion of matching alcohols, on the other hand, has little effect on lipid order [33]. These effects of alcohols may be rationalized as a balance between the energetic cost of increasing the membrane–water interface on one hand and the benefit of intermolecular interactions in the membrane core on the other. For a mismatched solute, the latter effect is weakened due to the looser packing in the inner part of the membrane. The contribution of the former effect then promotes lateral packing as reflected, for example, in the low apparent area of the inserted hexanol chains. Introduction of a matching alkanol, on the other hand, may not change the balance of core and interfacial interactions significantly and hence only exert a minor perturbation on lipid order.

In conclusion, the structural changes illustrated in Fig. 6 are in accord with those suggested on the basis of a comparative experimental study of the excess volumes of a homologous series of membrane partitioned alcohols [14]. In addition, the current results provide guidance for a critical analysis of the experimental data. Thus, the net volume change of about 4 cm^3 mol^{-1} found in both experiment and simulation is strongly affected by the comparably low density of pure hexanol (Table 2). More specifically, the conspicuous decrease in the Voronoi volume of hexanol upon transfer to the membrane (~9 cm^3 mol^{-1}, Table 3) reflects in part the rather loose packing in the neat alcohol. In other words, ΔV_{mix} is a balance of a positive contribution reflecting reorganization in the membrane and a negative contribution, which, at least in part, relies on the properties of pure alcohol. The latter is, of course, not pertinent to a discussion of mixed membranes, and ΔV_{mix} as defined by Eq. (2) may therefore provide a poor indication of the structural changes in the membrane. The Voronoi analysis suggested that the volume of the membrane core increased by ~14 cm^3 mol^{-1} of hexanol inserted, while the packing in the outer segments remained practically unchanged. If dilute aqueous hexanol is used as standard state (in stead of the neat organic liquid), the experimental volume change is about 11 cm^3 mol^{-1} [14], i.e. rather close to the value for the membrane derived from the Voronoi analysis. This accordance implies that the packing density of hexanol (expressed e.g. as its partial molar volume) is similar in water and membranes. We therefore suggest that experimental volume changes for membrane partitioning based on the dilute aqueous standard state (rather than the pure organic liquid) provide the most adequate basis in discussions of solute induced effects on the properties of lipid bilayers.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bpc.2006.07.005.

References

[1] H.G.L. Coster, D.R. Laver, The effect of benzyl alcohol and cholesterol on the acyl chain order and alkane solubility of bimolecular phosphatidylcholine membranes, Biochim. Biophys. Acta 861 (1986) 406–412.

[2] T.J. McIntosh, S.A. Simon, R.C. Macdonald, The organization of normal-alkanes in lipid bilayers, Biochim. Biophys. Acta 597 (1980) 445–463.

[3] M. Patra, E. Salonen, E. Terama, I. Vattulainen, R. Faller, B.W. Lee, J. Holopainen, M. Karttunen, Under the influence of alcohol: the effect of ethanol and methanol on lipid bilayers, Biophys. J. 90 (2006) 1121–1135.

[4] H.V. Ly, D.E. Block, M.L. Longo, Interfacial tension effect of ethanol on lipid bilayer rigidity, stability, and area/molecule: a micropipet aspiration approach, Langmuir 18 (2002) 8988–8995.

[5] H.V. Ly, M.L. Longo, Membrane thinning effect of ethanol: a micropipette aspiration approach, Abstr. Am. Chem. Soc. 221 (2001) U348-U348.

[6] H.V. Ly, M.L. Longo, The influence of short-chain alcohols on interfacial tension, mechanical properties, area/molecule, and permeability of fluid lipid bilayers, Biophys. J. 87 (2004) 1013–1033.

[7] D. Uhrikova, P. Balgavy, G. Rapp, Lipid bilayer thickness and surface area in lamellar phases of hydrated mixtures of dipalmitoylphosphatidylcholine and homologs of local anesthetic heptacaine, Mol. Cryst. Liq. Cryst. 373 (2002) 201–211.

[8] M.O. Jensen, O.G. Mouritsen, Lipids do influence protein function—the hydrophobic matching hypothesis revisited, Biochim. Biophys. Acta 1666 (2004) 205–226.

[9] R.G.W. Anderson, K. Jacobson, Cell biology—a role for lipid shells in targeting proteins to caveolae, rafts, and other lipid domains, Science 296 (2002) 1821–1825.

[10] R.S. Cantor, The lateral pressure profile in membranes: a physical mechanism of general anesthesia, Biochemistry 36 (1997) 2339–2344.

[11] R.S. Cantor, Solute modulation of conformational equilibria in intrinsic membrane proteins: apparent “cooperativity” without binding, Biophys. J. 77 (1999) 2643–2647.

[12] O.G. Mouritsen, M.J. Zuckermann, What’s so special about cholesterol? Lipids 39 (2004) 1101–1113.

[13] M. Nielsen, J. Thewalt, L. Miao, J.H. Ipsen, M. Bloom, M.J. Zuckermann, O.G. Mouritsen, Sterol evolution and the physics of membranes, Europhys. Lett. 52 (2000) 368–374.

[14] T.H. Aagaard, M.N. Kristensen, P. Westh, Packing properties of 1-alkanols and alkanes in a phospholipid membrane, Biophys. Chem. 119 (2006) 61–68.

[15] S.J. Marrink, H.J.C. Berendsen, Permeation process of small molecules across lipid membranes studied by molecular dynamics simulations, J. Phys. Chem. 100 (1996) 16729–16738.

[16] M. Kupiainen, E. Falck, S. Ollila, P. Niemela, A.A. Gurtovenko, M.T. Hyvonen, M. Patra, M. Karttunen, I. Vattulainen, Free volume properties of sphingomyelin, DMPC, DPPC, and PLPC bilayers, J. Comp. Theor. Nanosci. 2 (2005) 401–413.

[17] S.E. Feller, C.A. Brown, D.T. Nizza, K. Gawrisch, Nuclear overhauser enhancement spectroscopy cross-relaxation rates and ethanol distribution across membranes, Biophys. J. 82 (2002) 1396–1404.

[18] B.W. Lee, R. Faller, A.K. Sum, I. Vattulainen, M. Patra, M. Karttunen, Structural effects of small molecules on phospholipid bilayers investigated by molecular simulations, Fluid Phase Equilib. 225 (2004) 63–68.

[19] J.C. Phillips, R. Braun, W. Wang, J. Gumbart, E. Tajkhorshid, E. Villa, C. Chipot, R.D. Skeel, L. Kale, K. Schulten, Scalable molecular dynamics with NAMD, J. Comput. Chem. 26 (2005) 1781–1802.

[20] N. Foloppe, A.D. MacKerell, All-atom empirical force field for nucleic acids: I. Parameter optimization based on small molecule and condensed phase macromolecular target data, J. Comput. Chem. 21 (2000) 86–104.

[21] J. Sonne, F.Y. Hansen, G.H. Peters, Methodological problems in pressure profile calculations for lipid bilayers, J. Chem. Phys. 122 (2005).

[22] M.P. Allen, D.J. Tildesley, Computer Simulation of Liquids, Clarendon Press, Oxford, 1991.

[23] S.E. Feller, Y.H. Zhang, R.W. Pastor, Computer-simulation of liquid/liquid interfaces: 2. Surface-tension area dependence of a bilayer and monolayer, J. Chem. Phys. 103 (1995) 10267–10276.

[24] U. Essmann, L. Perera, M.L. Berkowitz, T. Darden, H. Lee, L.G. Pedersen, A smooth Particle Mesh Ewald method, J. Chem. Phys. 103 (1995) 8577–8593.

[25] J.D. Bernal, Bakerian lecture 1962—structure of liquids, Proc. R. Soc., A Math. Phys. Sci. 280 (1964) 299–312.

[26] F.M. Richards, Interpretation of protein structures—total volume, group volume distributions and packing density, J. Mol. Biol. 82 (1974) 1–14.

[27] J.M. Pope, L.W. Walker, D. Dubro, On the ordering of *n*-alkane and *n*-alcohol solutes in phospholipid-bilayer model membrane systems, Chem. Phys. Lipids 35 (1984) 259–277.

[28] J.F. Nagle, S. Tristram-Nagle, Structure of lipid bilayers, Biochim. Biophys. Acta 1469 (2000) 159–195.

[29] E. Lindahl, O. Edholm, Mesoscopic undulations and thickness fluctuations in lipid bilayers from molecular dynamics simulations, Biophys. J. 79 (2000) 426–433.

[30] J.L. Thewalt, R.J. Cushley, Phospholipid cholesterol membranes containing *n*-alkanols—a h-2-NMR study, Biochim. Biophys. Acta 905 (1987) 329–338.

[31] J.L. Thewalt, A.P. Tulloch, R.J. Cushley, A deuterium NMR-study of labeled normal-alkanol anesthetics in a model membrane, Chem. Phys. Lipids 39 (1986) 93–107.

[32] J.L. Thewalt, S.R. Wassall, H. Gorrissen, R.J. Cushley, Deuterium NMR-study of the effect of *n*-alkanol anesthetics on a model membrane system, Biochim. Biophys. Acta 817 (1985) 355–365.

[33] P.W. Westerman, J.M. Pope, N. Phonphok, J.W. Doane, D.W. Dubro, The interaction of normal-alkanols with lipid bilayer-membranes—a h-2-NMR study, Biochim. Biophys. Acta 939 (1988) 64–78.

[34] J.M. Pope, D. Dubro, J.W. Doane, P.W. Westerman, The ordering of benzyl alcohol and its influence on phospholipid order in bilayer-membranes, J. Am. Chem. Soc. 108 (1986) 5426–5433.

[35] J.M. Pope, D.W. Dubro, The interaction of normal-alkanes and normal-alcohols with lipid bilayer-membranes—a h-2-NMR study, Biochim. Biophys. Acta 858 (1986) 243–253.

[36] J. Ortega, Densities and thermal expansivities of hexanol isomers at moderate temperatures, J. Chem. Eng. Data 30 (1985) 5–7.

[37] J.F. Nagle, D.A. Wilkinson, Lecithin bilayers—density-measurements and molecular-interactions, Biophys. J. 23 (1978) 159–175.

[38] P. Westh, C. Trandum, Partitioning of small alcohols into dimyristoyl phosphatidylcholine (DMPC) membranes: volumetric properties, J. Phys. Chem., B 104 (2000) 11334–11341.

[39] P.F.F. Almeira, W.L.C. Vaz, in: R. Lipowsky, E. Sackmann (Eds.), Handbook of Biological Physics, Elsevier, Amsterdam, 1995, pp. 305–357.

[40] Y.K. Shin, U. Ewert, D.E. Budil, J.H. Freed, Microscopic versus macroscopic diffusion in model membranes by electron-spin-resonance spectral-spatial imaging, Biophys. J. 59 (1991) 950–957.

[41] W.L.C. Vaz, R.M. Clegg, D. Hallmann, Translational diffusion of lipids in liquid–crystalline phase phosphatidylcholine multibilayers—a comparison of experiment with theory, Biochemistry 24 (1985) 781–786.

[42] E.S. Rowe, F.L. Zhang, T.W. Leung, J.S. Parr, P.T. Guy, Thermodynamics of membrane partitioning for a series of *n*-alcohols determined by titration calorimetry: role of hydrophobic effects, Biochemistry 37 (1998) 2430–2440.

[43] H. Kamaya, S. Kaneshina, I. Ueda, Partition equilibrium of inhalation anesthetics and alcohols between water and membranes of phospholipids with varying acyl chain-lengths, Biochim. Biophys. Acta 646 (1981) 135–142.

[44] D.M. Fraser, L.C.M. Vangorkom, A. Watts, Partitioning behavior of 1-hexanol into lipid-membranes as studied by deuterium NMR-spectroscopy, Biochim. Biophys. Acta 1069 (1991) 53–60.

[45] G.R.A. Hunt, M. Kaszuba, The effect of normal-alcohols on vesicular permeability induced at the lipid phase-transition temperature—a h-1-NMR study, Chem. Phys. Lipids 51 (1989) 55–65.

[46] P.F.F. Almeida, W.L.C. Vaz, T.E. Thompson, Lateral diffusion in the liquid-phases of dimyristoylphosphatidylcholine cholesterol lipid bilayers—a free-volume analysis, Biochemistry 31 (1992) 6739–6747.
[47] S. Bhattacharya, S. Haldar, Interactions between cholesterol and lipids in bilayer membranes. Role of lipid headgroup and hydrocarbon chain-backbone linkage, Biochim. Biophys. Acta 1467 (2000) 39–53.
[48] J.A. Urbina, S. Pekerar, H.B. Le, J. Patterson, B. Montez, E. Oldfield, Molecular order and dynamics of phosphatidylcholine bilayer-membranes in the presence of cholesterol, ergosterol and lanosterol—a comparative-study using h-2-NMR, c-13-NMRr and p-31-NMR spectroscopy, Biochim. Biophys. Acta 1238 (1995) 163–176.
[49] H. Hauser, G.G. Shipley, Comparative structural aspects of cation binding to phosphatidylserine bilayers, Biochim. Biophys. Acta 813 (1985) 343–346.
[50] S. Keller, A. Tsamaloukas, H. Heerklotz, A quantitative model describing the selective solubilization of membrane domains, J. Am. Chem. Soc. 127 (2005) 11469–11476.
[51] L.L. Firestone, J.K. Alifimoff, K.W. Miller, Does general anesthetic-induced desensitization of the torpedo acetylcholine-receptor correlate with lipid disordering, Mol. Pharmacol. 46 (1994) 508–515.
[52] D.A. Driscoll, S. Samarasinghe, S. Adamy, J. Jonas, A. Jonas, Pressure effects on dipalmitoylphosphatidylcholine bilayers measured by h-2 nuclear-magnetic-resonance, Biochemistry 30 (1991) 3322–3327.