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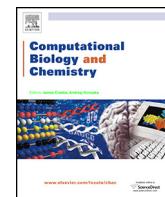


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Effect of acetone accumulation on structure and dynamics of lipid membranes studied by molecular dynamics simulations

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ABSTRACT

The modulation of the properties and function of cell membranes by small volatile substances is important for many biomedical applications. Despite available experimental results, molecular mechanisms of action of inhalants and organic solvents, such as acetone, on lipid membranes remain not well understood. To gain a better understanding of how acetone interacts with membranes, we have performed a series of molecular dynamics (MD) simulations of a POPC bilayer in aqueous solution in the presence of acetone, whose concentration was varied from 2.8 to 11.2 mol%. The MD simulations of passive distribution of acetone between a bulk water phase and a lipid bilayer show that acetone favors partitioning into the water-free region of the bilayer, located near the carbonyl groups of the phospholipids and at the beginning of the hydrocarbon core of the lipid membrane. Using MD umbrella sampling, we found that the permeability barrier of ~0.5 kcal/mol exists for acetone partitioning into the membrane. In addition, a Gibbs free energy profile of the acetone penetration across a bilayer demonstrates a favorable potential energy well of -3.6 kcal/mol, located at 15–16 Å from the bilayer center. The analysis of the structural and dynamics properties of the model membrane revealed that the POPC bilayer can tolerate the presence of acetone in the concentration range of 2.8–5.6 mol%. The accumulation of the higher acetone concentration of 11.2 mol% results, however, in drastic disordering of phospholipid packing and the increase in the membrane fluidity. The acetone molecules push the lipid heads apart and, hence, act as spacers in the headgroup region. This effect leads to the increase in the average headgroup area per molecule. In addition, the acyl tail region of the membrane also becomes less dense. We suggest, therefore, that the molecular mechanism of acetone action on the phospholipid bilayer has many common features with the effects of short chain alcohols, DMSO, and chloroform.

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1. Introduction

Inhalants such as organic solvents are breathable chemical vapors that can be inhaled to induce psychoactive or mind-altering toxic effects. Habit to take toxic volatile substances, such as glue, acetone, and cleaning agents, is also known as toxicomania disease. Although adequate epidemiological information is lacking, the apparently growing problem of inhalant abuse among young people has raised concerns in both affluent and developing nations. Despite an expanding body of knowledge and promising new avenues of research in fields of intoxication, dependence, and withdrawal, molecular mechanisms of action of volatile organic solvents on cell membranes remains poorly understood.

Over the past few years, several lines of evidence has accumulated indicating that binding and penetration of organic solvents, such as acetone, across biological membranes could lead to alterations in cellular membrane structure (Cantor, 1997; Tsai et al., 2001). The changes in membrane properties induced by acetone action could facilitate entrance of chemicals into and through lipid bilayers due to disruption of its permeability barrier (Tsai et al., 2001; Kezic and Nielsen, 2009). It has been shown by using of ratiometric fluorescent probes that the physico-chemical properties of cell membranes of rat olfactory mucos were modulated under the action of acetone (Posokhov et al., 2001). The observed changes in the cell membranes have been attributed to the accumulation of the acetone within the lipid bilayer (Posokhov et al., 2001; Posokhov, 2011). In addition, it has been found that the main phase transition temperature of DPPC vesicles, representing a model single-component membrane, was decreased with an increase in concentration of acetone (Kinoshita and Yamazaki, 1996). Using the ratio of excimer-to-monomer fluorescence of pyrene-PC, threshold concentrations of acetone for the phase transition at 20 °C

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have been found to be about 9.4% (v/v). The combination of X-ray diffraction and differential scanning calorimetry experiments has revealed that packing of the head groups at the surface of the DPPC bilayer was changed at higher acetone concentrations, so that the entire DPPC molecules were tend to form the crystallized phase in the plane of the bilayer (Kinoshita et al., 1997).

In many fields of membrane biophysics, in which experimental determination of molecular structures is difficult, molecular dynamics (MD) simulations have now become the powerful supplementary tools (Tieleman, 2006). MD simulations have successfully been applied for studies of binding, distribution, and transport of small organic compounds and ions within lipid membranes (Bemporad et al., 2005; Kyrychenko and Dyubko, 2008; Reigada, 2011). In such simulation studies, accurate MD force field parameters of the solute molecule are very critical. In this respect, the MD parameterizations capable of reproducing the properties and relative partition coefficients for pure acetone and its aqueous mixtures have intensively been studied and reported (Jorgensen et al., 1990; Ferrario et al., 1990). The earlier MD parameterization suggested by Jorgensen et al. has further been validated by combined spectroscopic and MD studies of binary mixtures of water with acetone over their entire range of compositions (Venables and Schmuttenmaer, 2000; Idrissi et al., 2001). In addition, the thermodynamics, aggregation behavior, and transport properties of aqueous mixtures of acetone have attracted considerable computational attention (Thompson, 1996; Weerasinghe and Smith, 2003; Liang et al., 2004). The reliability of the commonly used force fields for acetone has further been proved by computing its free energy of hydration using the multiconfiguration thermodynamic integration method (Helms and Wade, 1997; Reddy and Erion, 1999).

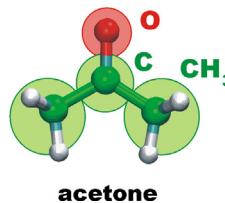
The purpose of the present work is to study interactions, adsorption, and accumulation of acetone in lipid membranes using MD simulations. We studied how multiple molecules of acetone, which were randomly distributed across bulk aqueous solution at the beginning of sampling, could bind and permeate into a POPC bilayer. In addition, we applied a joint refinement of unconstrained MD simulations of passive partitioning and umbrella sampling utilizing the potential of the mean constrained force calculations to study the favorable localization of acetone within a lipid bilayer. One of the goals of the present work was also to examine effects of low concentrations of acetone (2–6 mol%) on the membrane structure. Our MD simulations found that the presence of acetone in the concentration range 2.8–5.6 mol% could lead to small alterations in the structural and dynamics properties of the model membrane. These minor structural changes are often below the sensitivity of most convenient spectral methods; however, they might be detected by ultra-sensitive fluorescence techniques (Posokhov et al., 2001; Posokhov, 2011; Kinoshita and Yamazaki, 1996). In addition, we observed that accumulation of the high acetone concentration (11.2 mol%) in the bilayer could induce some decrease in the membrane thickness resulting in significant disordering of phospholipid packing and the increase in the membrane fluidity.

2. Methods

2.1. Molecular dynamics simulation setup

The force field parameters for acetone were taken from (Jorgensen et al., 1990) (Scheme 1). The model membrane was represented by 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) bilayer. The recently developed GROMOS 53A6L force field (Poger et al., 2010) was used to model the phospholipid bilayer. These parameters, which form the part of the GROMOS 54A7 force field (Schmid et al., 2011), reproduce a broad range of membrane properties (Poger and Mark, 2010, 2012). In the 53A6L force field,

United-Atom Model Force Field Parameters



site	q, e	$\sigma, \text{\AA}$	$\epsilon, \text{kcal/mol}$
O	-0.424	2.96	0.210
C	0.300	3.75	0.105
CH ₃	0.062	3.91	0.160

Scheme 1. An united-atom model and non-bonded force field parameters for acetone: two acetone methyl groups CH₃ with non-polar hydrogen atoms were treated as the united atom site. Electric charges (q) and non-bonded Lennard-Jones parameters (σ , ϵ) were adopted from Jorgensen et al. (1990).

all carbon atoms of CH₂ and CH₃ groups with non-polar hydrogen atoms were treated as united atoms. The initial configuration of an equilibrated POPC bilayer composed of 128 lipids was used from our previous studies (Kyrychenko et al., 2010, 2011). The Simple Point Charge (SPC) model (Hermans et al., 1984) was used for water.

The MD simulations were carried out at the constant number of particles, constant pressure of $P=1$ atm, and the constant temperature $T=303.15$ K (NPT ensemble). Three-dimensional periodic boundary conditions were applied with the z axis lying along a direction normal to the bilayer. The pressure was controlled semi-isotropically, so that the x-y and z dimensions of the simulation box were allowed to fluctuate independently from each other, keeping the total pressure constant. Thus, during MD simulations, the membrane area and thickness were, therefore, free to adjust under the NPT condition. The reference temperature and pressure were kept constant using the Berendsen weak coupling scheme (Berendsen et al., 1984) with a coupling constant of $\tau_T=0.1$ ps for the temperature coupling and $\tau_{P(x-y)}=\tau_{P(z)}=1.0$ ps for the pressure coupling. Electrostatic interactions were simulated with the particle mesh Ewald (PME) (Darden et al., 1993) approach using the long-range cutoff of 1.4 nm. The cutoff distance of Lennard-Jones interactions was also equal to 1.4 nm. All bond lengths were kept constant using the LINCS routine (Hess et al., 1997). The MD integration time step was 2 fs. The MD simulations were carried out using the GROMACS set of programs, version 4.5.5 (Van Der Spoel et al., 2005). Molecular graphics and visualization were performed using VMD 1.8.6 (Humphrey et al., 1996).

3. Results and discussion

3.1. Interactions with a model membrane

Our main goal is to characterize the effect of acetone accumulation on the bilayer properties and to correlate these changes with our experimental observations (Posokhov et al., 2001; Posokhov, 2011). For this reason, the development of a novel MD model and the force field for acetone would be well beyond the scope of our manuscript. For acetone molecules, we used the well-documented force field validated for acetone partitioning between two solvent phases (Jorgensen et al., 1990; Reddy and Erion, 1999). Among a variety of the MD force field available for lipid bilayers, our choice of the GROMOS 53A6L force field for the bilayer (Poger and Mark, 2012) was dictated by our interest in validating of its accuracy for partitioning thermodynamics calculations.

To study the distribution and favorable localization of acetone in a lipid membrane, we first applied unconstrained MD simulations based on passive distribution of acetone molecules between bulk water and a POPC bilayer. Four membrane systems were considered: (A) a pure POPC (128 lipids) bilayer in water, the system A containing 50 (B), 100 (C), and 200 (D) acetone molecules added, which corresponds to molar percent (mol%) of acetone in

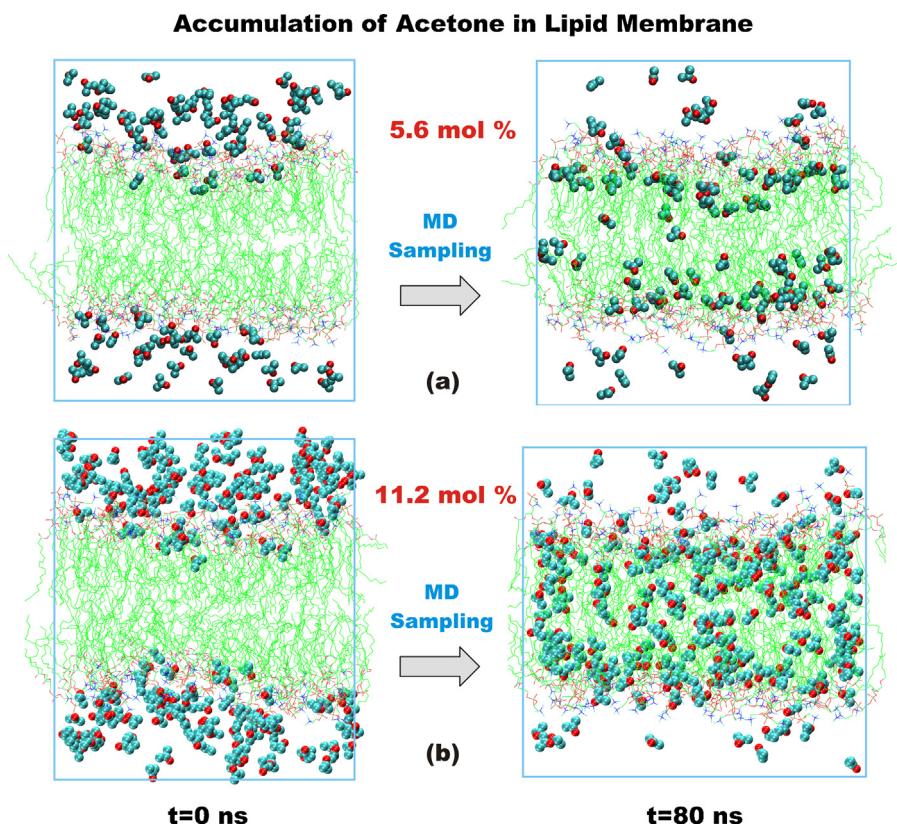


Fig. 1. Snapshots of MD simulations of acetone partitioning into the POPC bilayer are shown for two acetone concentrations of 5.6 mol% (a) and 11.2 mol% (b), respectively. The MD snapshots are shown for the initial ($t=0$ ns) and final ($t=80$ ns) simulation times. The POPC bilayer is drawn by color-coded sticks. The hydrophobic acyl-chain region of the bilayer is shown by green. The acetone molecules are drawn in van der Waals representation. For clarity, water molecules are not shown. (For interpretation of the references to color in figure legend, the reader is referred to the web version of the article.)

the bilayer to be 2.8, 5.6, and 11.2 mol%, respectively. In the initial configurations, all the acetone molecules were placed into bulk water in the vacancies left by the removal of the corresponding number of water molecules. To remove high energy contacts, the systems were equilibrated at NPT conditions for 1 ns. After the equilibration period, the acetone molecules were subject to free, thermally-driven diffusion within the simulation cell. Interactions between the acetone molecules and the phospholipid bilayer, including binding, partitioning, and accumulation in the bilayer, were studied using a series of unconstrained MD simulations for a simulation time of 80 ns. The simulations were run independently for each acetone/POPC/water system.

The initial configurations ($t=0$ ns) of two acetone/POPC systems containing 5.6 and 11.2 mol% of acetone are shown in Fig. 1 (left). It has been shown that free, unconstrained MD sampling of passive diffusion and partitioning of solutes into a bilayer is slow and, therefore, can occur in a time scale of 40–60 ns (Kyrychenko and Dyubko, 2008; Kyrychenko and Waluk, 2008). Fig. 1 (right) also shows snapshots of the partitioning dynamics of acetone in the POPC bilayer taken at the end of the MD sampling ($t=80$ ns). As can be noted, the majority of the solute molecules become bound to the interfacial region of the POPC bilayer and become buried deeper across the hydrophobic region of the bilayer, as shown in Fig. 1 (right).

3.2. Localization within a POPC bilayer

A typical MD snapshot of position and orientation of the acetone molecules in the POPC bilayer is given in Fig. 2. The analysis of the favorable localization of acetone molecules shows that their carbonyl oxygen atoms are preferably oriented toward the polar

membrane interface, whereas the hydrophobic CH_3 groups are faced inside the lipid membrane. Due to thermal diffusion and continuous exchange of acetone molecules between the bulk water phase and the lipid bilayer, orientation of a $\text{C}=\text{O}$ vector of acetone

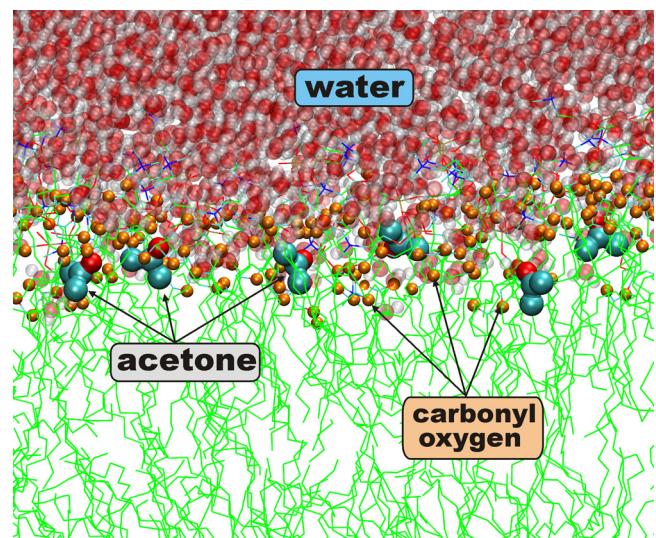


Fig. 2. Location and binding modes of acetone molecules in the POPC bilayer: the MD snapshot shows that the acetone molecules favors residing within the bilayer region located near the carbonyl groups of the phospholipids and at the beginning of the hydrocarbon core of the lipid membrane (green). The acetone molecules are drawn in van der Waals representation. The carbonyl oxygen atoms of the lipid molecules are shown in orange. (For interpretation of the references to color in figure legend, the reader is referred to the web version of the article.)

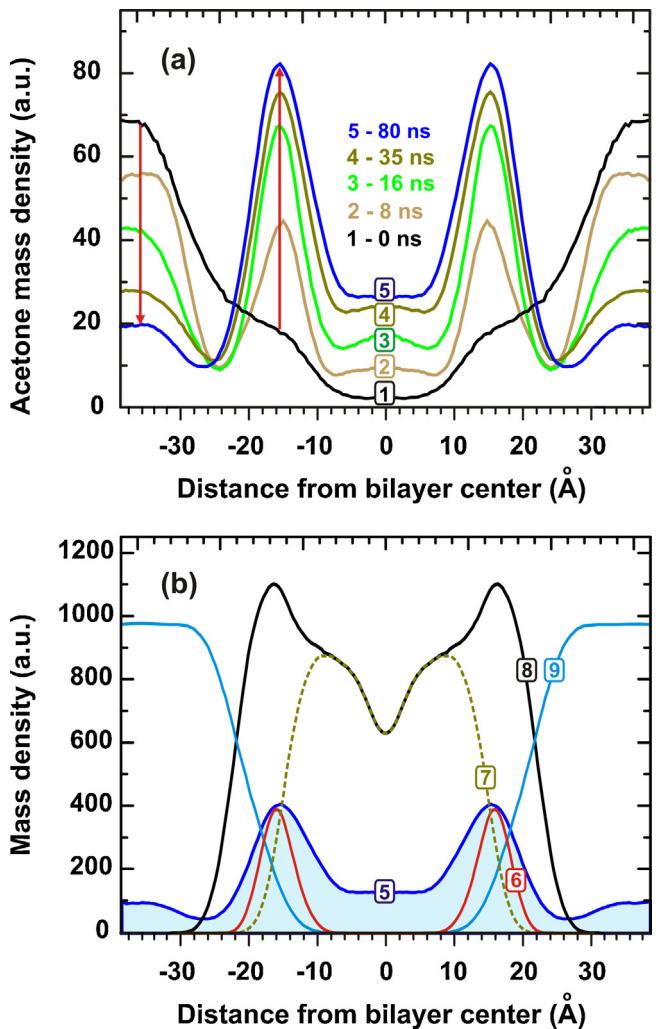


Fig. 3. (a) MD simulations of adsorption of acetone by the POPC bilayer (the concentration of acetone in the membrane was 5.6 mol%). Transverse mass density of acetone was calculated from the center of the bilayer and plotted as a function of the MD simulation time. To monitor the adsorption dynamics, the acetone density was averaged over the time intervals: 1 (0 ns), 2 (0–8 ns), 3 (8–16 ns), 4 (16–35 ns), 5 (40–80 ns). The red arrows indicate the decrease in the acetone population in the aqueous phase accompanying with the increase in the acetone fraction within the bilayer. (b) The equilibrium distribution of acetone is superimposed with the mass profiles of the whole bilayer and its individual components. The distribution profile of acetone is shown by curve 5 (see panel (a) for more details). The mass density of acetone is compared with the densities of the carbonyl (6) and acyl (7) moieties of the lipids, as well as the overall mass profile of the model membrane (8) and water (9), respectively. (For interpretation of the references to color in figure legend, the reader is referred to the web version of the article.)

with respect to the bilayer normal shows very broad and almost isotropic distribution (not shown).

To evaluate the distribution of acetone within the POPC bilayer quantitatively, transverse profiles of mass density of various molecular components were estimated. The position of all atoms in the acetone/membrane systems were calculated and averaged with respect to the Z axis, normal to the bilayer. Using the symmetry of the bilayer, the mass density profiles were also averaged over the two, upper and lower, leaflets of the bilayer.

Fig. 3a shows the mass distribution of acetone within the POPC bilayer averaged over discrete time intervals. The distribution is shown for the acetone concentration of 5.6 mol%. At the beginning of the MD sampling, acetone was distributed within the aqueous phase (curve 1, the peak density located at 32–38 Å from the bilayer center). As can be seen from Fig. 3a, during the first 16 ns, the

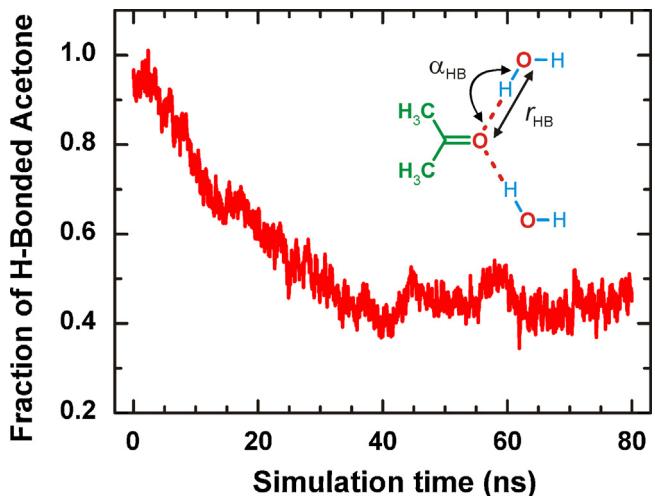


Fig. 4. Structure and geometry criteria (distance r_{HB} and angle α_{HB}) of hydrogen bonds formed between acetone and two water molecules. Time evolution of the fraction of hydrogen-bonded acetone complexes was monitored by MD simulations for the acetone/POPC/water system (100/128/4458, containing 5.6 mol% of acetone). A decrease in acetone-to-water hydrogen bonding suggests that acetone partitioning deep into the water-free region of the bilayer.

majority of the acetone molecules were rapidly re-distributed from water into the interfacial region and the hydrocarbon core of the bilayer. After 35 ns of the MD sampling, the acetone mass density becomes converged to some constant values indicating that the system reached the equilibrium state. To estimate the equilibrium distribution across the bilayer, the mass density of acetone was averaged over the last time period of 40–80 ns, as shown by curve 5 in Fig. 3a.

Fig. 3b shows the mass density profiles of the simulated system, including acetone, the POPC bilayer and water. At the equilibrium state, the major population of acetone was found to reside at 15–16 Å from the bilayer center. This region is located near the carbonyl groups of the phospholipids and at the beginning of the hydrocarbon core of the membrane. At the end of the MD sampling (when the system reached the equilibrium state) some minor population of acetone could still be observed in bulk water outside the membrane.

3.3. H-bonding of acetone with water

To investigate the convergence for the adsorption and accumulation of acetone in the model bilayer, we monitored the number of hydrogen bonds formed between acetone and water molecules as a function of MD simulation time. Acetone is able to form two equivalent hydrogen bonds with two water molecules. In the both hydrogen bonds, the acetone carbonyl oxygen acts as a hydrogen bond acceptor and an hydrogen atom of water molecules participates as a hydrogen bond donor, as shown schematically in insert of Fig. 4. The presence of hydrogen bonds between the acetone and water molecules was established using two geometry criteria: (1) the distance between the donor and acceptor is $r_i \leq r_{\text{HB}} = 3.5 \text{ \AA}$; (2) the angle made by the donor, the hydrogen, and the acceptor atom is $\alpha_i \leq \alpha_{\text{HB}} = 0^\circ \pm 30^\circ$. The probability (P_{HB}) for the formation of a hydrogen bond between acetone and water molecules was estimated as the average number of hydrogen bonds in each 100 ps time frame over the whole period of the MD sampling.

In Fig. 4, the time evolution of P_{HB} is shown for 80 ns of the MD sampling. At $t=0$ ns, the majority of the acetone molecules exist as hydrogen-bonded complexes with water molecules. During MD simulations, P_{HB} shows a rapid decrease from almost complete hydrogen bonded (94%, at $t=0$ ns) to values below 25% at

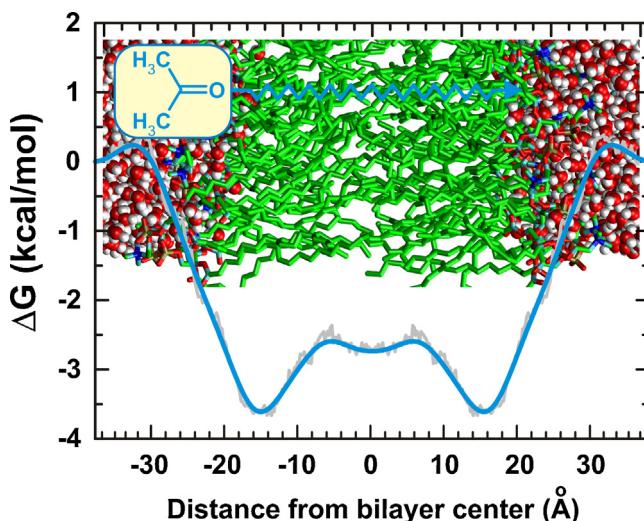


Fig. 5. A Gibbs free energy profile of the permeability of acetone across the POPC bilayer was estimated by MD simulations of the potential of mean constraint force. The PMF was calculated from the biased distributions using the weighted histogram analysis method. The permeability profile was set to zero in bulk water. For the visualization purpose, the PMF is schematically superimposed on a MD snapshot of a pure POPC bilayer.

$t = 40\text{--}80\text{ ns}$. A decrease in a number of hydrogen bonds formed between acetone and water molecules indicates that acetone leaves the bulk water phase and moves into the water-free region of the lipid bilayer. The results of the above hydrogen bonding behavior are in good agreement with the results of the accumulation dynamics of acetone monitored with the mass density, as shown in Fig. 3a. The partition of the acetone molecules from water deep within the hydrophobic, water-free region of the POPC bilayer results in a significant decrease in P_{HB} of acetone-to-water hydrogen bonding.

Importantly, Fig. 4 shows that, in presence of the POPC bilayer, the number of H-bonds decrease rapidly during the first 30 ns reaching some plateau at 40–80 ns. After 40 ns this parameter was fluctuating around some average value of 45 indicating that the system reached the equilibrium state so that a rate of acetone adsorption by the bilayer become equal to the reverse process of acetone desorption back to the bulk water phase.

3.4. Permeability across a POPC bilayer

A Gibbs free energy profile for the permeability of acetone across the POPC bilayer was estimated by the method of the potential of mean force (Kumar et al., 1992). This approach is based on a series of constrained MD samplings in which a solute molecule is transferred across a bilayer under action of an external force referred to as the potential of mean force (PMF). The umbrella sampling scheme used for computing the PMF is shown in Fig. 5. To gather additional statistics, two acetone molecules were sampled simultaneously, so that when the first molecule reaches the center of a bilayer, the second molecule appears in the bulk water.

To calculate the PMF profile, we carried out a series of MD runs, in which the center of mass of each of two acetone molecules was restrained with respect to the center of mass of the POPC bilayer at distance d_c . During the PMF simulations, distance d_c was varied within a range starting from 38 Å (bulk water) and ending at 0 Å (the center of the bilayer). A harmonic restraint of $1500\text{ kJ mol}^{-1}\text{ nm}^{-2}$ was applied to the distance between the center of mass of each acetone molecule and the center of mass of a POPC bilayer. A total of 26 restraint points were sampled in the direction normal to the bilayer. Each simulation was run for 2 ns. The potential of mean force was

calculated from the biased distributions using the weighted histogram analysis method (Kumar et al., 1992).

Fig. 5 shows the PMF of a penetration profile of acetone across a POPC bilayer. The PMF profile for acetone shows a favorable free-energy well of -3.6 kcal/mol which is centered on 15.6 Å from the center of a bilayer (Fig. 5). The ΔG profile suggests that to enter the bilayer acetone needs to cross the very low free energy barrier of 0.5 kcal/mol. When acetone approaches the minimum of the free energy well, it experiences a low ($\sim 1.2\text{ kcal/mol}$) barrier to reach the middle of the bilayer. The PMF profile minimum found at 15.6 Å agrees well with the distribution peak of acetone in a POPC bilayer estimated with the unbiased passive MD simulations (Fig. 3a and b).

It has been shown by several experimental studies that acetone could facilitate entrance of other chemicals into and through lipid bilayers due to disruption of its permeability barrier (Tsai et al., 2001). It should, therefore, be noted that the free energy barrier of 0.5 kcal/mol observed for partitioning of acetone itself is potentially dependent on some MD parameters, such as force field parameters for lipid molecules and charge distribution on a solute molecule. Recent MD studies of partitioning thermodynamics of small solute molecule have also shown an important role of molecular polarizability in modulating the free energy profiles for their crossing through lipid bilayers (Vorobyov et al., 2012; Jambeck and Lyubartsev, 2013).

3.5. Perturbation of bilayer structure

To study effects of acetone on the structural and dynamic properties of the model membrane, we first simulated the pure POPC bilayer in water. The structure of the pure bilayer was characterized by the density distribution of its individual functional groups, such as choline, phosphate and carbonyl groups, as well as a glycerol backbone and acyl chains of the phospholipids. In addition, the MD-estimated bulk properties of the bilayer, such as the area per lipid, bilayer thickness and acyl chain order parameters, were found to agree with experiment. These parameters were also consistent with those reported in previous benchmark MD simulations of the same POPC bilayer (Kyrychenko et al., 2010, 2011).

It has been demonstrated that interaction of the model membrane with small organic solutes, such as sugars and alcohols could often lead to the alteration of the bilayer properties (Bemporad et al., 2005; Gurtovenko and Anwar, 2009). Therefore, we first considered the effects of acetone on the bulk structure of the membrane as a function of its concentration. We found that upon adsorption and accumulation of acetone, the POPC membrane expands in X-Y sizes, which leads to systematic increases of the area per lipid from 62.4 Å (pure POPC), to 63.2 Å (2.8 mol%), 66.8 Å (5.6 mol%), and 75.9 (11.2 mol%), respectively.

To consider changes in the structure of the membrane along the normal Z, the mass density profile of the POPC bilayer, estimated by the MD simulations in the absence and in the presence of acetone, were plotted from the center of the bilayer as shown in Fig. 6a. As can be seen, the presence of 5.6 mol% of acetone results in very small changes in bulk membrane structure. The small decrease in the membrane thickness was observed when the acetone concentration reached 11.2 mol%. To study the membrane hydration, the mass density profiles of water is also plotted. As can be noted, the low acetone content results in very small changes in the penetration profile of water across the bilayer. However, at the higher acetone concentration of 11.2 mol%, water molecules penetrated deeper within the membrane. Our MD simulations show that acetone favors to reside in the region around and below the carbonyl groups of the phospholipids. The effect of acetone on the shape and position of the density profile of the lipid carbonyls was, therefore, compared in Fig. 6a. It can be concluded that the accumulation of

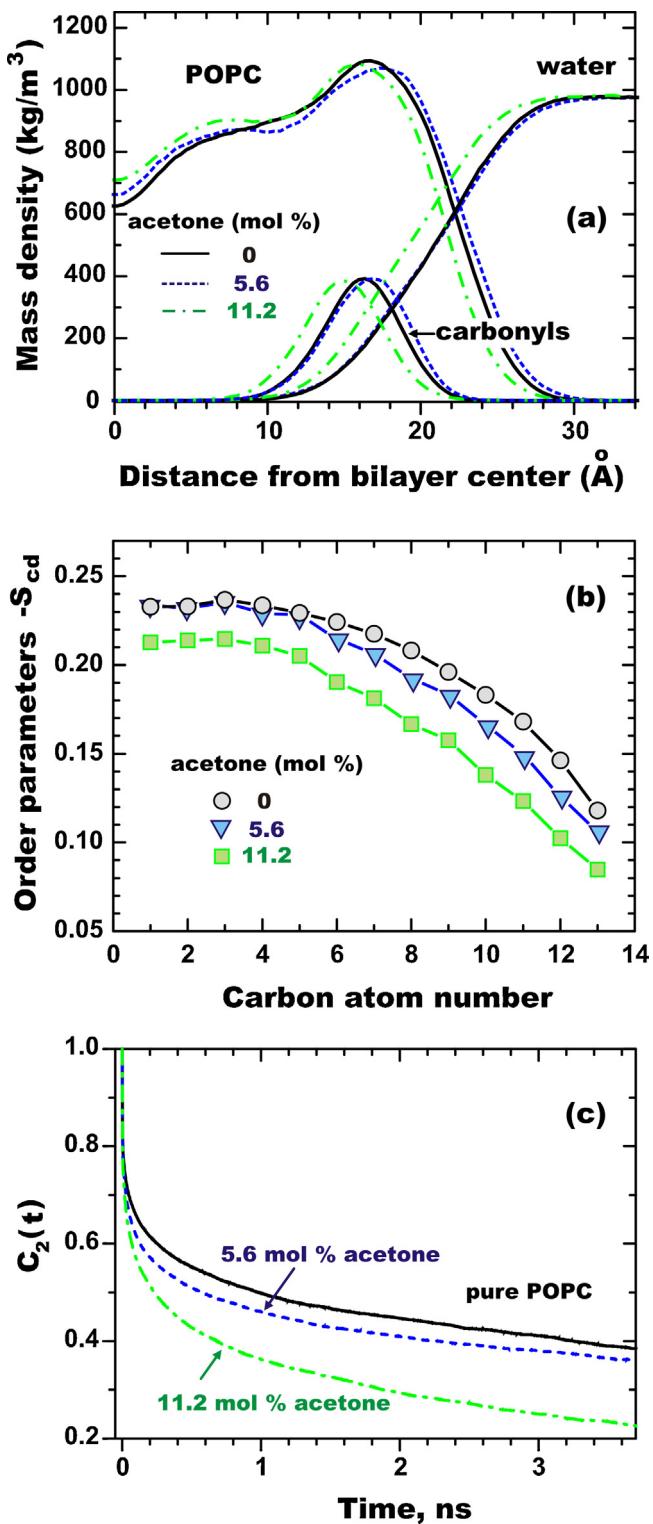


Fig. 6. Effect of acetone accumulation on the bilayer structure: the mass density, lipid order parameters, and rotation autocorrelation functions were estimated in the absence and in the presence of acetone, respectively. For each system, the properties were calculated for the last 60–80 ns of the MD sampling periods. (a) The mass density of POPC, the carbonyl moieties of the lipids and water are plotted for the pure POPC bilayer (solid lines), and for the systems containing 5.6 mol% (dashed) and 11.2 mol% (dash-dotted) of acetone, respectively. (b) Lipid tail deuterium order parameter S_{cd} calculated for the *sn*-2 acyl chain of the POPC bilayer. (c) Rotation autocorrelation functions for the carbonyl groups of the POPC lipids. The accumulation of acetone results in the systematic decrease in the overall correlation time of the lipid carbonyl moiety indicating that acetone partitioning into the membrane is accompanied by a disordering of the phospholipid packing and the increase in the lipid mobility.

acetone at the low concentration in the model bilayer causes rather small overall perturbations of its bulk structure and the thickness.

To characterize average orientation of fatty lipid chains with respect to the bilayer normal the order parameters of the acyl chains are often used. It is important to note that lipid acyl chain order parameters can be directly obtained by NMR measurements from deuterium NMR quadrupole splitting. In addition to experiment, such distributions can also be evaluated by MD simulations (Vermeer et al., 2007).

Lipid order parameters S_{cd} are a measure for the orientation mobility of the C–D bond and evaluated through the order parameter tensor (Eq. (1)):

$$S_{\alpha\beta} = \frac{1}{2} \left\langle 3 \cos \Theta_\alpha \cos \Theta_\beta - \delta_{\alpha\beta} \right\rangle \quad (1)$$

in which Θ_α is the angle between the α th molecular axis and the bilayer normal. Due to molecular symmetry, the order parameter S_{cd} can be obtained through $S_{cd} = -S_{zz}/2$.

It has been shown that the acyl chain order parameters $-S_{cd}$ were correlated with membrane rigidity, area expansion modulus as well as the elastic properties, such as compressibility (Vermeer et al., 2007). Therefore, the order parameters can also be used to study the effect of acetone on the structure of the model bilayer. Fig. 6b shows the order parameters for the pure POPC bilayer (in the absence of acetone) and in the presence of 5.6 and 11.2 mol% of acetone, respectively. In the case of 2.8 mol% of acetone, the order parameters were found to be very similar to those of the pure model membrane (not shown). As can be seen, the presence of 5.6 mol% of acetone leads to the systematic lowering of the acyl chain order parameters. At the higher acetone content of 11.2 mol%, drastic disordering of the POPC bilayer was observed.

Due to thermal diffusion, the lipid molecules in the membrane are a subject for rotational reorientation and diffusive motions about the membrane normal, originating from flexibility of the lipid chains. Many movements occur on different timescales: rotation around chemical bonds and *trans/gauche* isomerisations (picoseconds), rotation (axial diffusion) around the lipid axis (nanoseconds), lateral diffusion (microseconds), and flip-flop across the bilayer (milliseconds). Therefore, another very important characteristic of the dynamics of lipid molecules in membranes is the rotational motion (Essmann and Berkowitz, 1999). Because of the nature of the lipid bilayer, the overall motion can be characterized by a rotation around Z axis parallel to the bilayer normal (Kyrychenko and Ladokhin, 2013). The reorientation dynamics of the individual fragments of the lipid molecule can be calculated from a rotation autocorrelation function (RACF) $\langle C_2(t) \rangle$ (Eq. (2)).

$$\langle C_2(t) \rangle = \frac{2}{5} \left\langle \frac{3 \cos^2 (\cos (\Theta(t)) - 1)}{2} \right\rangle \quad (2)$$

Typical autocorrelation function for the rotational motion of the carbonyl groups of the POPC lipids is shown in Fig. 6c. As can be seen, a fast initial decay of the correlation functions is followed by slower exponential decays. The decrease in the correlation time observed upon accumulation of acetone in the membrane support a overall molecular picture in which partitioning of solutes into membranes is accompanied by a disordering of the phospholipids (Westh and Trandum, 2000).

3.6. Comparison with other organic solvents

It has been found both experimentally and computationally that the addition of organic solvents, such as alcohols (Cantor, 1997; Patra et al., 2006; Terama et al., 2008; Gurtovenko and Anwar, 2009; Pillman and Blanchard, 2010), chloroform (Reigada, 2011), or DMSO (Anchordoguy et al., 1992; Gurtovenko and Anwar, 2007;

Dabkowska et al., 2011; Hughes et al., 2012) destabilizes the structure of model membranes so that they become thinner and their area per lipid headgroup increases. In contrast, sugar molecules such as trehalose or sucrose (which are often used as cryoprotective agents) tend to stabilize the structure of the bilayer because they bridge adjacent lipid headgroups (Sum et al., 2003; Pereira et al., 2004; Pereira and Hünenberger, 2008).

Chanda and Bandyopadhyay have observed that, at the concentration of 12.5 mol%, ethanol molecules preferentially occupy regions near the DMPC bilayer interface, in agreement with NMR data (Chanda and Bandyopadhyay, 2004). At this ethanol concentration, only small changes in bilayer structure and in the lipid hydrocarbon chain conformations have been observed. These authors have found, however, that at higher ethanol concentrations (1:1 lipid-to-ethanol ratio), the ethanol molecules exhibit a preference to occupy regions near the upper part of the lipid acyl chains and the phosphocholine headgroups (Chanda and Bandyopadhyay, 2006). Their MD simulations have revealed that the phosphocholine headgroup dipoles ($P^- \rightarrow N^+$) of the lipids prefer to orient more toward the aqueous layer in the presence of ethanol. It was also noticed that the ethanol molecules modify the dynamic properties of lipids as well as the water molecules in the hydration layer of the lipid headgroups (Chanda and Bandyopadhyay, 2006). Moreover, Terama et al. have demonstrated that ethanol partitioning is considerably more favorable in unsaturated bilayers, which are often characterized by their more disordered nature compared to their saturated counterparts (Terama et al., 2008). These authors have simulated varying ethanol concentrations and suggested that the partitioning of ethanol is concentration dependent (Terama et al., 2008). Finally, Gurtovenko and Anwar have also considered the concentration dependence of ethanol on the structure of the POPC membrane. They revealed that at concentrations below the threshold value of ~12 mol% ethanol induces expansion of the membrane, accompanied by a drop in the membrane thickness as well as disordering and enhanced interdigitation of lipid acyl chains (Gurtovenko and Anwar, 2009). At these conditions, the bilayer structure of the membrane was still maintained. Importantly, the changes in membrane structure become more pronounced with the increase in ethanol concentration so that above the threshold concentration the appearance of multiple transient defects in the lipid/water interface was detected (Gurtovenko and Anwar, 2009).

Effects of DMSO on the structure of model lipid membranes have been reported to be similar in many aspects to those of alcohols (Gurtovenko and Anwar, 2007; Dabkowska et al., 2013; Notman et al., 2006): at low concentrations of DMSO causes the membrane to expand in the plane of the membrane while decreases its thickness (Hughes et al., 2012). Above a critical concentration, DMSO induces the spontaneous formation of pores in the membrane, and if the concentration was increased further, then the drastic destabilization of bilayer structure was often observed. The molecular basis for the action of DMSO on the membranes has been suggested to be due to the fact that DMSO molecules favor to reside just below the headgroup region and, therefore, act as spacers/pivots that enhance lipid-lipid separation (Notman et al., 2006). Therefore, DMSO makes the membrane significantly floppy which facilitates membrane fusion processes, reduce the barrier to molecular transport, and assist pore formation (Notman et al., 2006). These features are directly related to the capability of DMSO in cryopreservation and membrane permeability enhancement.

It has recently been shown that due to the diffusive nature of xenon molecules, xenon accumulation in the POPC bilayer could also lead to a decrease in the orientational order of the lipid tails, an increase in the area and volume per lipid molecule, and an increase in the diffusivity of lipid molecules (Yamamoto et al., 2012). Therefore, this effect of xenon on the membrane structure has been

suggested as the possible mechanism of the pressure reversal of general anesthesia (Yamamoto et al., 2012).

The above discussed actions of small anesthetic molecules, such as alcohols and DMSO, on the membranes have many features which are similar to those observed in the case of our MD study of acetone. We can therefore conclude that the molecular mechanism of action of small amphiphilic molecules on the phospholipid membranes may have a common feature similar for acetone, short chain alcohols, DMSO, and chloroform. They all partition into the densest region of a membrane and perturb the bilayer interacyl chain spacing and/or organization, leading to the increase in the local mobility of the lipid molecules inside the bilayer (Pillman and Blanchard, 2010) and an increase in the water content of the membrane (Westh and Trandum, 2000).

It is also instructive to mention that effect of acetone accumulation on the membrane properties may have important impact for drug-membrane interactions. The traditional view that drugs interact with membranes predominantly via specific binding with membrane proteins has recently been questioned and reviewed. The recent review of MD studies have demonstrated that more and more data support the perspective according which some drugs interact directly with lipids and, hence, modulating effect of acetone is crucial for these processes (Cramariuc et al., 2012; Notman and Anwar, 2013).

4. Concluding remarks

In this study, we have presented results of MD simulations of the POPC lipid bilayer containing different concentrations of acetone ranging from 2.6 to 11.2 mol%. Our study has several goals: first, our objective was to simulate free partitioning of acetone molecules into the lipid bilayer to identify a region of its favorable localization in the membrane. Second, we wanted to characterize the influence of acetone on the structure and dynamics of a lipid membrane by exploring the effects of acetone as a function of its concentration. The MD results of the acetone/POPC systems were compared with the pure POPC bilayer and available experimental data (Posokhov, 2011).

To study the location of the acetone molecules within the bilayer and its effect on the structure and dynamics of the lipid molecules we used the well-recommended force field for acetone (Jorgenson et al., 1990). Our study demonstrates that below a critical concentration (in a range of 2.8–5.6 mol%) the acetone molecules prefer to occupy a region located near carbonyl groups of the phospholipids and at the beginning of the hydrocarbon core of the lipid membrane (at 15–16 Å from the bilayer center, as shown in Fig. 3b). In addition to the free, unconstrained MD simulations of acetone accumulation within the membrane we used the MD umbrella sampling procedure to estimate the Gibbs free energy for transfer of acetone from bulk water to the center of the POPC bilayer. The minimum of the energy profile of the penetration across a bilayer was found to be $\Delta G = -3.6$ kcal/mol. The ΔG minimum was located 15–16 Å from the bilayer center, which agree well with the favorable localization estimated alternatively using the free MD sampling. Moreover, the results of the MD calculations of the potential of mean force suggest that the entrance and partitioning of acetone deep into the membrane occurs through the low permeability barrier of ~0.5 kcal/mol.

The overall structure of the bilayer was modified by acetone only slightly when acetone was added to the model membrane at concentrations 2.8–5.6 mol% which is below the critical concentration. Above the critical concentration (at 11.2 mol%) the accumulation of acetone within the membrane induces its significant in-plane expansion (X-Y sizes), accompanied with the decrease in the membrane thickness (Fig. 6). These findings are in line with X-ray

diffraction and differential scanning calorimetry experiments performed on unilamellar lipid vesicles (Kinoshita et al., 1997).

Our MD simulations show that the acetone molecules tend to reside near the carbonyl groups of the lipid molecules. As a consequence, the acetone molecules push the lipid heads apart and, hence, act as spacers at the headgroup level, so that the average headgroup area per molecule increases. The acyl tail region of the membrane also becomes less dense, which enables the tails of the neighboring lipids to expand into an effectively larger volume. The overall effect of acetone accumulation is that acetone causes the bilayer to become less rigid, leading to the increase in local lipid mobility within the membrane. The actions of acetone on the membranes have, therefore, many similar features of those of alcohols and DMSO. We can conclude that the molecular mechanism of action of small amphiphilic molecules on the phospholipid membranes may have the common features.

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