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Dynamical and structural properties of charged and uncharged lidocaine in a lipid bilayer

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Abstract

Molecular dynamics computer simulations have been performed to investigate dynamical and structural properties of a lidocaine local anesthetic. Both charged and uncharged forms of the lidocaine molecule were investigated. Properties such as membrane area per lipid, diffusion, mass density, bilayer penetration and order parameters have been examined. An analysis of the lidocaine interaction with the lipid surrounding according to a simple mean field theory has also been performed. Almost all examined properties were found to depend on which of the two forms of lidocaine, charged or uncharged, is studied. The overall picture is a rather static behavior determined by the lipids for the charged molecules and more mobile situation of the uncharged form with higher diffusion and lower orientational and positional order.

Keywords: Lidocaine; Lipid membrane; Molecular dynamics

1. Introduction

Lidocaine-family drugs are widely used as local anesthetics in medical treatment to prevent or relieve pain. The anesthetic action of lidocaine is based on its ability to block Na^+ voltage-gated ion channels in the nervous system [1–3]. The specific molecular mechanism of this action is still poorly understood. This effect is often ascribed to a direct binding of lidocaine to Na^+ channel [4–6]. Significant amount of evidence has been also presented for the influence of lidocaine on membrane properties [7–9] which may also trigger the conductivity of ion channels.

In aqueous solution lidocaine molecules exist as a mixture of uncharged and positively charged (protonated) species depending on the pH value of the solution (the pK value of lidocaine is estimated to be between 7.5 and 9) [10]. Inside membranes the balance is shifted in favor of the uncharged species [11]. It is assumed that the anesthetic action of lidocaine (as well as other local anesthetics) is related to its protonated form [4]. The role of the uncharged form may also be important: due to its higher hydrophobicity, the uncharged form has higher membrane-water partition coefficient [11] and thus can faster penetrate and diffuse in membranes.

Experimental results providing information about molecular interactions of lidocaine with surrounding lipid membrane are rather fragmented. Fourier-transform infrared spectroscopy (FTIR) studies [12] have shown that local anesthetics are positioned in the membrane-water interface region and may compete for the water molecules with lipid headgroups. The presence of lidocaine leads also to a more fluid character of the lipid membrane and decreases transition temperature to the gel phase [9,12]. EPR and NMR spectroscopy studies of the uncharged lidocaine in phosphatidylcholine membrane have been reported [13,14] and a model for a preferential location of lidocaine near the glycerol region has been suggested.

In this paper we report, to our knowledge, the first computer simulation study of structural and dynamical properties of lidocaine molecules in a lipid bilayer. During the last decade, molecular dynamics simulations have been extensively used to study many properties of lipid membranes [15–20] including cases where some other components (cholesterol [21], methanol or ethanol [22]) where added. Molecular simulations enable us

Abbreviations: DMPC, Dimyristoylphosphatidylcholine; EPR, Electron paramagnetic resonance; FTIR, Fourier-transform infrared spectroscopy; MSD, Mean square displacement; MFT, Mean field theory; NMR, Nucleic magnetic resonance; PME, Particle mesh Ewald; RDF, Radial distribution function; SPC, Simple point charge.

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to follow every detail of the molecular motion and thus provide a unique insight into machinery behind all molecular processes. In this work we address questions relevant for general understanding of interaction of lidocaine molecules with surrounding lipids: location of lidocaine in membrane, diffusion, hydration, formation of hydrogen bonds. Also, orientational order of lidocaine and lipid molecules is investigated and analyzed using a simple mean field theory. We consider both charged and uncharged forms of lidocaine molecules and compare their properties.

2. Simulation details

Two different lipid bilayer systems, each consisting of 128 (64 × 2) dimyristoylphosphatidylcholine (DMPC) lipids, 3655 water molecules and 12 lidocaine molecules were simulated. The system with charged lidocaine contained also 12 chloride ions to balance the charge. In addition to the systems containing lidocaine molecules, one system containing a pure fully hydrated bilayer was simulated as a reference.

For DMPC lipids, we used the united atom model for hydrocarbon groups. Previous studies of phospholipid bilayers have shown that both united atom and all-atom force fields yield generally similar results, while the united atom model provides more than three-fold saving of the computer time due to a significantly lower number of atoms. The lipid force field parameters for bonded and non-bonded interactions as well as atomic partial charges are based on the GROMOS force field [16], with modification by Berger et al. [17]. The DMPC molecular structure with the names of atoms referenced in the text is shown in Fig. 1. For water the simple point charge (SPC) model was used [23].

As for the lipids, the united atom model was used to describe lidocaine (except the polar H atom in the charged form). Though all-atomic model, including possibly polarization effects, may be important for correct presentation of pi-interactions in the aromatic ring, we did not include these effects in order to keep consistency with the used lipid model. Similar united atom model was previously used in DPPC–cholesterol simulations [21]. The lidocaine molecular structure and interaction parameters were prepared using the *prodrg* server [24]. The interaction parameters were taken from the GROMOS force field included into GROMACS simulation package [25]. The partial atom charges for the both forms were assigned according to the charges of similar molecular fragments found in the GROMOS force field, with modifications based on Hartree–Fock quantum chemical calculations carried out for optimized geometries of these molecules using

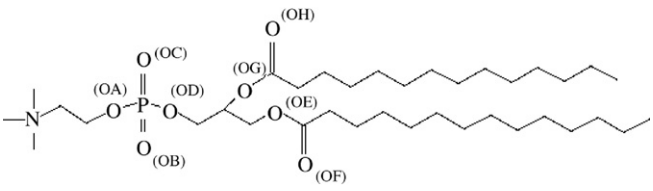

Fig. 1. The DMPC molecule with atom names used in the text.

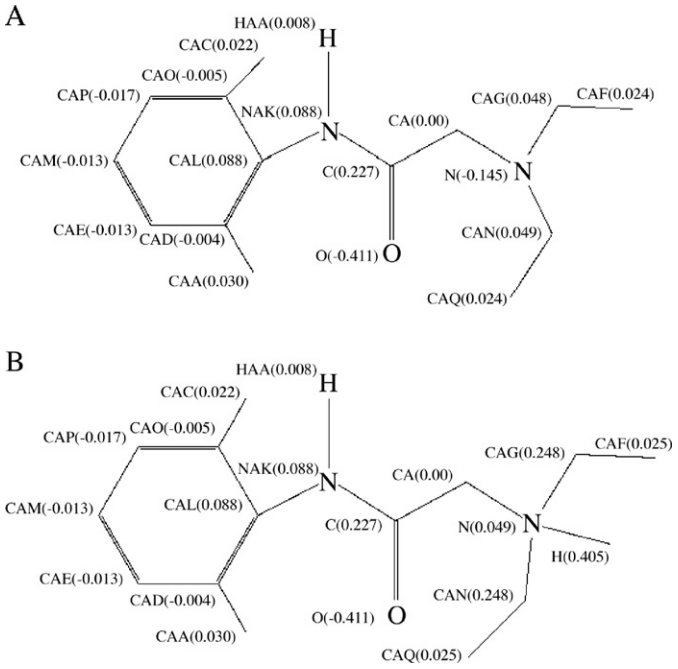

Fig. 2. Uncharged (A) and charged (B) lidocaine with atom names used in the text.

6–31* basis set. The lidocaine molecules with partial charges and atom names are shown in Fig. 2.

The starting configuration for the lipids was taken from our previous simulation [20], where the lipids were in the liquid crystalline phase. The lidocaine molecules were inserted in close vicinity to the membrane surface and with an equal number of molecules on both sides of the bilayer. Then water molecules were added in necessary amount not overlapping with the already placed components. The systems were energy minimized and in each case a 1 ns pre-equilibration run with isotropic cell fluctuations was carried out. The starting configuration of the reference neat bilayer system (including water) was used from the previous simulation [20] without modifications.

All simulations were preformed with the Gromacs simulation package v. 3.2 [25]. All bond lengths were kept constant at their equilibrium values using the LINCS algorithm [26]. The time step was set to 2 fs.

The temperature in the simulated systems was set to 310 K and was regulated separately for lipids and for water/lidocaine using the Nose-Hoover thermostat [27]. This temperature corresponds to the liquid crystalline structure of the neat DMPC bilayer. It is also known that addition of local anesthetics decreases the temperature of transition to the gel phase [12] that is why the bilayer remains in the liquid crystalline phase even upon addition of lidocaine. The pressure was set to 1 atm and was regulated by the Parrinello-Rahman barostat [28] semi-anisotropically with two degrees of freedom: one along the Z dimension and another in the XY plane. The relaxation constants were set to 0.4 ps for the thermostat and 2.0 ps for the barostat.

The long range electrostatic forces were treated by using the Particle Mesh Ewald (PME) algorithm [29]. In a number of methodological studies it was demonstrated that the Ewald

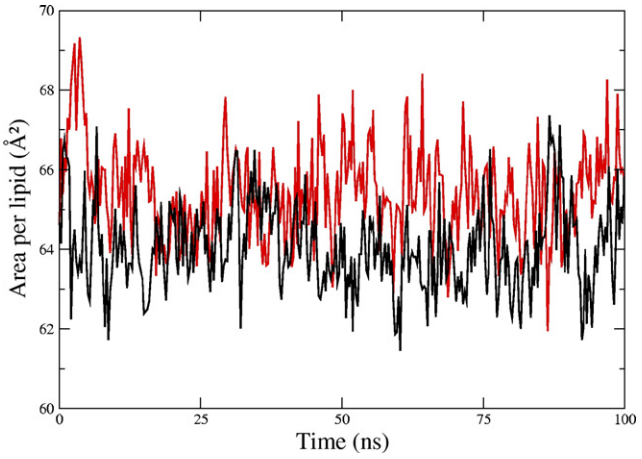

Fig. 3. The area per lipid as a function of time. Charged lidocaine: red, uncharged lidocaine: black.

method provides a better stability of the simulation results upon the change of the system size, and a better agreement with experiments for several important parameters compared to simulations with a simple electrostatic cutoff [30–32].

The cutoff radius for the Lennard–Jones interactions was set to 10 Å. The dispersion correction from the interactions outside the cutoff was included into energy and pressure. A neighbor list scheme was used with the list update frequency at every 10-th time step. The eventual center of mass motion of the system was removed every 500 fs.

The systems containing lidocaine were simulated for 100 ns from the starting conditions described above. The last 50 ns were used for trajectory analysis. Since the reference system was taken from a previous well equilibrated simulation (at slightly different temperature), it was simulated 50 ns from which the last 45 ns were used for trajectory analysis.

3. Results and discussion

All the lidocaine molecules entered the bilayer several nanoseconds after the simulation start. This is not surprising since the experimentally measured logarithm of membrane-water partition coefficient log*P* is 2.4 for the neutral lidocaine and 1.22 for the charged form [11]. Note that in the simulations the affinity of lidocaine to the membrane phase may be somewhat overestimated since previous calculations of octanol–water log*P* for uncharged lidocaine (though with different force field) showed a value of 3.3 which is one unit higher compared with the experiment [33].

3.1. Area per lipid

The time evolution of the area per lipid is a very fundamental property of lamellar bilayer systems. Here we use the area per lipid to monitor equilibration of the simulated systems. In simulations the area per lipid is defined as the average box length in the *X*-dimension multiplied by the average box length in the *Y*-dimension divided by the number of lipids in one layer.

The time evolution of areas per lipid for charged and uncharged lidocaine are shown in Fig. 3. No visible trends are observed. Block averaging over the last 50 ns of the trajectory showed no drift in the evolution of the area per lipid for the simulated systems and consequently the systems were considered to be in equilibrium. The results demonstrate that all the system were in the liquid crystalline phase which is also supported by observations of other properties.

The average areas are given in Table 1. These results show a small increase of the area per lipid compared to a neat DMPC bilayer, 64.2 Å^2, simulated with the same setup and force field.

The increase of the area per lipid with addition of lidocaine has an obvious explanation: lidocaine molecules occupy some space between the lipids. A more interesting result is that for the system with charged lidocaine the average area per lipid, reported in Table 1 is 1 Å^2 smaller than in the system with the uncharged lidocaine and almost coincide with the average area in the reference bilayer. Statistical analysis (*t*-test) shows that this difference is significant on the 0.05 level. The difference in lipid area can be attributed to differences in the molecular orientation and positioning in the bilayer of charged and uncharged lidocaine, which will be discussed below.

3.2. Mass density

Mass densities of the charged and uncharged lidocaine as well as of water and DMPC molecules are presented in Fig. 4. The noticeable difference in the distribution of the uncharged lidocaine molecules on the different sides of the bilayer is due to the fact that the simulation time is too short in order to sample the lidocaine distribution accurately. It turned out that the uncharged lidocaine molecules jump sometimes from one side of the bilayer to the other, and during our 50 ns averaging time the average concentration of lidocaine in the two layers was unequal due to natural fluctuations. To examine the implications of this effect on the results, we investigated short intervals (up to 10 ns) of the uncharged lidocaine trajectory during which the number of molecules was equal on both sides of the bilayer. No significant difference in the shape or positioning of the distribution was found. Furthermore, the right and left parts of the uncharged lidocaine distribution in Fig. 4 differ mostly by a

Table 1
Summary of simulation results discussed in the text

Property	Uncharged lidocaine	Charged lidocaine
Area per lipid (Å^2)[a]	65.4(0.16)	64.4(0.12)
Distance from the lidocaine density maxima to the bilayer center (Å)	8.5 (1)	11 (1)
Lidocaine lateral diffusion (10^{-8} cm^2/s)	36.6(0.5)	8.0(0.4)
Lipid lateral diffusion (10^{-8} cm^2/s)[b]	6.8 (0.4)	6.2 (0.4)
Average crossing time (ns)	3.3(1)	–
Average crossing frequency (ns)	100 (6)	–
Order parameter of N-CAL vector	−0.18(0.02)	0.45(0.03)
Order parameter of C-CAM vector	−0.11(0.02)	0.48(0.03)

The statistical error is given in parenthesis.
[a] Reference bilayer: 64.2 (0.2) Å^2.
[b] Reference bilayer: 6.7 (0.5) 10^{-8} cm^2/s.

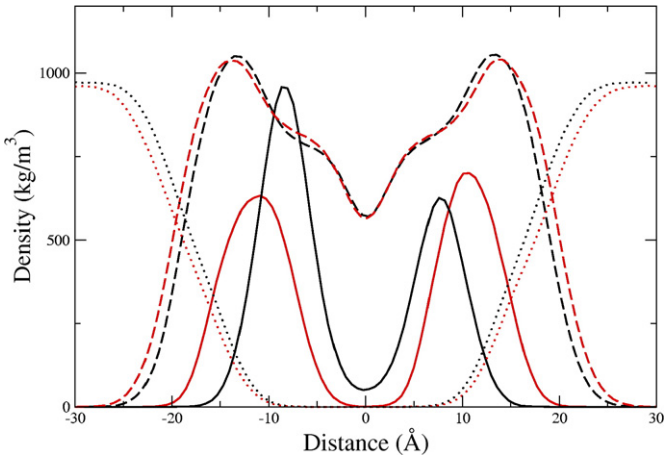

Fig. 4. The mass density dependence on the distance from the bilayer center. System with charged lidocaine: red, with uncharged lidocaine: black. Mass density of lidocaine: solid lines, lipids: dashed lines and water: dotted lines.

constant factor equal to the ratio of the average number of lidocaine molecules in each side of the bilayer. Note also, that in terms of free energy the relative difference in the two peaks of the uncharged lidocaine distribution is less than 0.5 kT.

In Fig. 4 it can be seen that the charged and uncharged lidocaine molecules have preference for different depths in the bilayer. The charged molecule has its maxima at 11 Å and the uncharged at 8.5 Å from the bilayer center. This corresponds to a situation where the charged molecules were located close to the polar headgroup of the lipids and the uncharged molecules located in the region of the ester groups and upper part of the lipid tails. This is in agreement with previous hypotheses regarding the distribution of polar and apolar anesthetics [8] and more recent EPR and NMR measurements of the uncharged lidocaine [13].

The probability of finding the charged lidocaine molecule is essentially zero over a distance of 2 Å around the bilayer center, while the uncharged molecules show a nonzero distribution in the same region. One can estimate from the Born model of ionic hydration [34] an effective free energy barrier of about 60 kJ/mol (25 kT units) for the charged lidocaine to cross the bilayer (assuming a spherical molecule of volume equal to that of lidocaine and $\varepsilon=2$ dielectric permittivity in the middle of the membrane). This indicates that uncharged lidocaine molecules are able to penetrate to the middle of lipid bilayer whereas charged lidocaine molecules are not.

Looking at the mass density for the water it can be noted that the two different forms of lidocaine have a small influence on the water distribution. The charged lidocaine has a tendency to pull the water out of the bilayer whereas the uncharged lidocaine pulls water into the bilayer. The effect is quite small ($\sim$1 Å) but interesting when taken together with the mass density for the lidocaine molecule. The charged molecules seems to be positioned in such a way to expel bound water from the lipid headgroup while the uncharged lidocaine is distributed lower into the bilayer causing the lipid area to increase and, as a consequence, allowing the water molecules to penetrate deeper into the bilayer.

3.3. Diffusion

Differences in translational diffusion of charged and uncharged lidocaine as well as for the lipids was analyzed from their root mean square displacement (MSD). The diffusion of the molecular center of mass was calculated from the Einstein relation:

$$D = \lim \frac{1}{2n}\frac{\mathrm{d}}{\mathrm{d}t}\langle|\Delta r(t)|^2\rangle \quad (1)$$

where $\langle|\Delta r(t)|^2\rangle$ is the MSD in the XY plane during time t starting from the initial time t_0, n is the dimensionally of the system ($n=2$ for the lateral diffusion) and D is the diffusion coefficient. Averaging is taken over all the molecules and over all initial times t_0 acceptable for the given observation time t. Here the diffusion coefficient was calculated from a linear fit of the MSD vs. time plot in the range 10–20 ns.

In Table 1 the lateral diffusion coefficients for charged and uncharged lidocaine as well as for the lipids are presented. From the results it can be seen that the diffusion of the charged molecule is almost a factor of five slower than the uncharged. An interesting observation is that the lateral diffusion of the charged lidocaine is only slightly higher than the lipid diffusion. This indicates that the charged molecules are associated to the lipids and diffuse with them, while the uncharged species are most likely free to move between the lipids.

The effect of lidocaine on the diffusion of lipids is small. When charged lidocaine is present, a small decrease of the diffusion coefficient can be noted.

3.4. Crossing events

In order to get another insight into the motion of the lidocaine inside the lipid bilayer we have analyzed the center of mass movement parallel to the normal of the bilayer as a function of time. From the results shown in Fig. 5 it is obvious that the charged lidocaine molecules never cross the bilayer while the uncharged lidocaine sometimes does so. Taking the number of crossing events, defined as lidocaine translation from one side of the bilayer to the other and staying there, and dividing it by the analyzed time interval, we get an average rate of crossing events in the simulated system as one per 8.3 ns. This corresponds to an average time about 100 ns for each lidocaine molecule to stay within one of the monolayers. The mean time spent in crossing the bilayer for uncharged lidocaine is estimated to 3 ns.

Not all crossing events are successful: for example, the molecule marked by a pink line in Fig. 5A crossed the middle plane at about 78 ns but returned after less than nanosecond. We did not count such events as a transition. Looking again at Fig. 5A it can be noted that the uncharged lidocaine molecules reside in the bilayer at a depth just where the mass density of the DMPC molecule has a plateau after its maximum in the headgroup region. To be able to move from one side of the

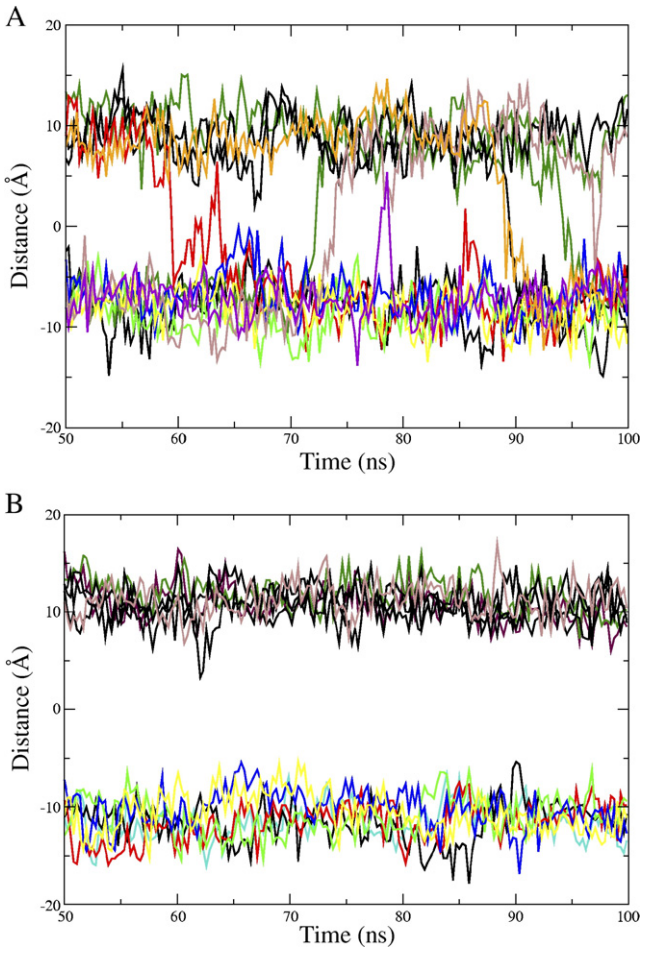

Fig. 5. The distances from the bilayer center to the center of mass of each lidocaine molecule as functions of time. (A) uncharged lidocaine; (B) charged lidocaine. Different molecules are represented by different colors.

bilayer to the other the lidocaine molecule has to cross the density minima, called lipid through. By visual inspection of the trajectory the mechanism for a successful crossing event seems to depend on the orientation of the lidocaine molecule. While approaching the center (approximately 5 Å from the middle plane) the lidocaine molecule adopts an orientation parallel to the bilayer normal, which is also parallel to the lipid tails (note that a preferential orientation of the uncharged lidocaine is parallel to the membrane surface, see below). To be able to translate over to the other leaflet the molecule has to maintain this parallel orientation until it makes contact with the lipids in the other leaflet. It seems that necessity to adopt and maintain orientation parallel to the bilayer normal in the region of lipid through creates a potential barrier making crossing events rare enough.

3.5. Hydrogen bonds and radial distribution functions

In previous experimental studies [12] anesthetics have shown to be able to form hydrogen bonds to different atoms in the polar headgroup and water as well as to compete with lipids for hydrogen bonding with water. This has also been suggested as a possible mechanism for the action of molecules with anesthetic effect [12]. Here we have investigated hydrogen bonding between the lidocaine molecules and the lipids, between water and lipids as well as between water and lidocaine. We define the existence of a hydrogen bond by a distance between acceptor and hydrogen being less than 2.5 Å and the angle between acceptor-hydrogen and hydrogen-donor vectors being less than 30°. The results are summarized in Table 2a.

It is clear from Table 2a that it is about ten times more common for the charged lidocaine molecule to form hydrogen bonds to DMPC lipids compared to the uncharged lidocaine. For the uncharged lidocaine molecule the hydrogen bonding to the lipids is virtually absent: an uncharged lidocaine molecule forms hydrogen bonds about 1% of the time (each timeframe has this kind of hydrogen bond in 12% of cases). For the charged lidocaine molecules the probability to form a hydrogen bond is about 15%. About 4% of them form hydrogen bonds to the phosphate moiety and 11% to the lipid ester oxygens. The dominating hydrogen bonding acceptor on the lipids is clearly the double bonded oxygen.

The results for hydrogen bonding between lidocaine and water also show a large difference between charged and uncharged lidocaine. An uncharged molecule has a hydrogen bond to water about 12% of the time while the charged molecule forms an average of 1.1 hydrogen bonds. For the uncharged molecule almost all hydrogen bonds are formed to the double bonded oxygen. For the charged molecule the dominating hydrogen bonding site is the hydrogen on the N-group, which in fact always has one hydrogen-bound water. Another hydrogen bond is sometimes formed with NAK or O atoms, and this happens more frequently compared to the uncharged lidocaine.

Table 2a
Average number of hydrogen bonds for lidocaine

	Total bonds per timeframe	Bonds per lidocaine
Uncharged lidocaine–DMPC		
Total	0.12	0.01
OA+OD	0.006	0.0005
OB+OC	0.002	0.0002
OE+OG	0.065	0.005
OF+OH	0.050	0.004
Charged lidocaine–DMPC		
Total	1.8	0.15
OA+OD	0.28	0.024
OB+OC	0.14	0.011
OE+OG	0.32	0.027
OF+OH	1.06	0.088
Uncharged lidocaine–H_2O		
Total	1.42	0.12
N	0.003	0.0003
NAK	0.011	0.001
O	1.40	0.12
Charged lidocaine–H_2O		
Total	12.76	1.06
N	12.0	1.0
NAK	0.20	0.017
O	0.56	0.046

The difference in a number of hydrogen bonds between charged and uncharged lidocaine has a clear origin: the charged form has an additional hydrogen bond donor, which is very active in formation of hydrogen bonds due to the strong polarity of the corresponding NH group.

The fact that the charged lidocaine molecules form more hydrogen bonds than the uncharged ones and at the same time tend to pull water out of the bilayer is interesting. This can be attributed to the positioning of the lidocaine molecules in the bilayer. The charged lidocaine is positioned in close vicinity to the DMPC headgroup and has often its polar part in contact with the polar ester or phosphate group. It squeezes out water from this region leading to a lower density near the ester groups. The uncharged lidocaine molecules are positioned deeper into the bilayer, near the upper part of lipid tails. This leads to slightly larger distance between the lipids (which is also manifested in the larger area per lipid) and creates additional space in between lipid heads, into which additional water molecules can penetrate.

Further insight into hydration of a lidocaine molecule in the bilayer can be obtained by looking at Fig. 6 where radial distribution functions (RDF) for the oxygen water to different atom sites in the charged and uncharged lidocaine are presented.

From the RDF plots it is clear that the charged lidocaine N-atom has a water molecule positioned at a very defined distance of 2.5 Å. This group also has a quite well defined second hydration shell at a distance of 4.6 Å. Two other RDF for the charged lidocaine shows smaller maxima: at 3 Å for O atom and at 5 Å for the NAK atom. The explanation for the long distance of the peak in the RDF for the NAK atom is probably that it originates from the same water molecules responsible for the hydration of the O atom which has its RDF maximum at a distance of 3 Å and which has hydrogen bonded water, according to Table 2a, in about 5% of the cases. Taken together with the distance between the O and NAK groups this makes a plausible explanation. The RDF for corresponding groups for the uncharged lidocaine show, in accordance with the hydrogen bonding results, almost no sign of hydration.

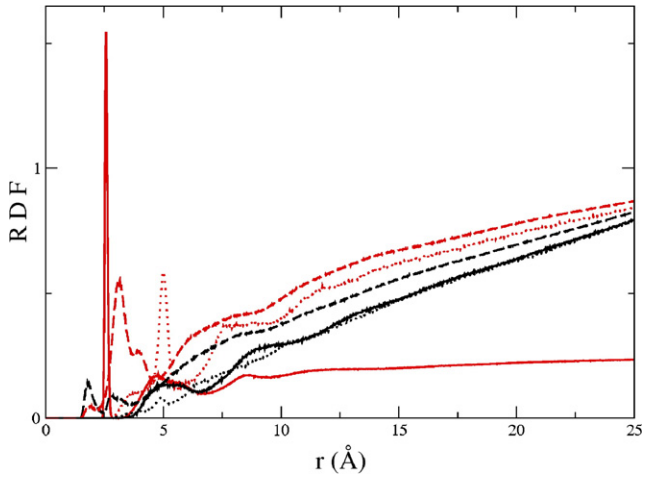

Fig. 6. Radial distribution functions for the water oxygen around some lidocaine atoms: N (solid lines), NAK (dotted lines) and O (dashed lines). Charged lidocaine: red, uncharged lidocaine: black. For the N atom, the RDF plot was scaled down four times to fit the figure.

Table 2b
Average number of hydrogen bonds for DMPC

	Total	Bonds per DMPC
H_2O–DMPC with uncharged lidocaine		
Total	810	6.31
OA,OD	72	0.56
OB,OC	423	3.30
OE,OG	40	0.31
OF,OH	275	2.15
H_2O–DMPC with charged lidocaine		
Total	785	6.13
OA,OD	67.5	0.53
OB,OC	411	3.21
OE,OG	38.7	0.30
OF,OH	267	2.09
H_2O–DMPC reference bilayer		
Total	807	6.3
OA,OD	70	0.55
OB,OC	421	3.29
OE,OG	41.6	0.32
OF,OH	275	2.15

When looking at the results for the hydrogen bonding between water and DMPC (Table 2b), there seems to be only minor effect of uncharged lidocaine on the total number of such bonds compared to the case of reference membrane without added lidocaine. The charged lidocaine causes however some decrease in water— DMPC hydrogen bonding, most noticeable for the phosphate moiety (OC and OD atoms). This effect can be explained by competition for hydrated water molecules between charged lidocaine and lipids. The effect is relatively small with respect to the total number of hydrogen bonds in the system at the given (relatively small) concentration of lidocaine molecules.

3.6. Orientational order and mean field theory analysis

To investigate the orientational order of lidocaine molecules in the bilayer we have calculated the second rank order parameters for the molecular long axis defined as:

$$S = \frac{1}{2}\langle 3\cos^2\theta - 1\rangle \quad (2)$$

where θ is the angle between a vector fixed in the molecular frame and the bilayer normal, here assumed to be parallel to the z-axis of the simulation box, and averaging $\langle\ldots\rangle$ is taken over all lidocaine molecules and time frames. Two vectors, each defined by two atoms, were considered for the representation of the principal molecular axis: i) N-CAL and ii) C-CAM.

The order parameters for charged and uncharged lidocaine long axis are collected in Table 1. The charged lidocaine shows rather high order parameters, 0.45 and 0.48 for the two vectors, whereas the order parameters for the uncharged lidocaine are significantly lower and negative: −0.18 and −0.11. This indicates that the charged lidocaine adopts an orientation parallel to the bilayer normal. Visual inspection of the trajectories shows that the

aromatic ring is always oriented to the alkyl interior of the bilayer and the protonated nitrogen (N) is positioned in the headgroup region. From the order parameter of the uncharged lidocaine it can be inferred that the molecule has a preference for the orientation orthogonal to the bilayer normal. When approaching the low density bilayer center region the molecule sometimes orients parallel to the bilayer normal and has the possibility to diffuse to the other leaflet. However for the "typical" location of lidocaine close to the ester groups, the order parameter should be even more negative. So while the charged lidocaine is rather restricted in its position and orientation, the uncharged lidocaine molecule exhibits a significant degree of translational and orientational motion.

To evaluate quantitatively the difference in interaction for the two forms of lidocaine molecules with the lipid environment, the mean field theory (MFT) was employed. In fact, there is a large number of MFTs, with the common assumption that a molecule experiences an average interaction field created by the neighbor molecules. The MFT analysis is often used in interpretations of experimental NMR parameters determined in partially ordered systems [35,36]. The general details of the MFT in liquid crystals are described in [37], here we give a short recapitulation of the theory.

The orientational distribution $f^{i}(\theta)$ of the molecular specie i is related to the potential of mean torque $U^{i}(\theta)$ by:

$$f^{i}(\theta) = \frac{1}{Q^{i}} \exp[-U^{i}(\theta)RT] \tag{3}$$

where Q^{i} is the orientational partition function given by:

$$Q^{i} = \int \exp(-U^{i}(\theta)/RT)\sin\theta \mathrm{d}\theta. \tag{4}$$

According to the Maier–Saupe theory for binary mixtures of cylindrically symmetric molecules [38] the potential of mean torque can be written as:

$$U^{i}(\theta) = -\varepsilon^{i}\frac{1}{2}(3\cos^{2}\theta - 1). \tag{5}$$

Here ε^{i} is the interaction parameter, which for diluted solution of lidocaine in DMPC can be defined as:

$$\varepsilon^{\mathrm{LID}} = u^{\mathrm{LID-DMPC}} S^{\mathrm{DMPC}} \tag{6}$$

where $u^{\mathrm{LID-DMPC}} \equiv u$ is the average lidocaine–DMPC interaction parameter, and S^{DMPC} is the order parameter of DMPC lipids. Since the molecular interaction is determined by the pair potential, the potential of mean torque and therefore u reflects the symmetry of the liquid crystalline phase and the solute molecule. By assuming a spherical distribution of intermolecular vectors defined by the radial distribution function, u can, in principle, be evaluated analytically from a pair potential [35]. This assumption has been previously tested in MD computer simulations of thermotropic liquid crystals [39–41]. In the present work, the orientational distribution function derived from the trajectory and displayed in Fig. 7 was numerically fitted to Eq. (3) which enables the extraction of the interaction parameters.

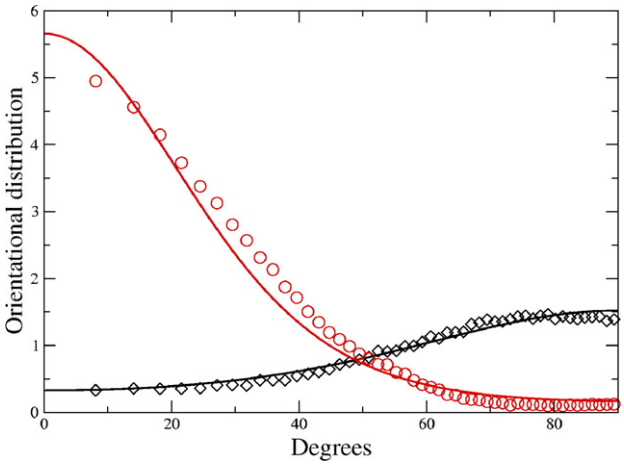

Fig. 7. Distribution $f(\theta)$ for molecular N-CAL vector. Charged lidocaine: red; uncharged lidocaine: black. Points are simulated data and lines are fitting according to the mean field theory.

The order parameters corresponding to the C13–C26 vector in DMPC was estimated to $S^{\mathrm{DMPC}} = 0.5$ both in charged and uncharged lidocaine systems. From the fitting procedure of the orientational distribution functions in Fig. 7 the following average interaction parameters were derived: $u = 12.0$ and $u = -5.3$ kJ/mol for the charged and uncharged lidocaine, respectively. The distribution functions plotted using these parameters are also included in Fig. 7. The agreement between the fitted and computed (from the trajectory) distributions is clearly satisfactory, indicating that the simple mean field theory approach provides an adequate description of the orientational order in these systems. The opposite signs of the interaction parameters reflect the orientation of the charged/uncharged lidocaine molecules with respect to the director and should not be interpreted in terms of repulsive/attractive forces. The significantly higher value of the interaction parameter for the charged lidocaine shows its higher average interaction with the surrounding compared with the uncharged lidocaine. The charged lidocaine molecules have, due to strong electrostatic interactions, preference for the headgroup region of the lipids, and so a higher value of u. On the other hand, the uncharged lidocaine interacts weaker with the surrounding, which is also in accordance with its ability to move quite freely in the lipid part of the bilayer.

4. Conclusions

In this article we have examined the behavior of charged and uncharged lidocaine in a lipid DMPC bilayer. Significant differences for the two types of molecules were found for all examined properties. The overall picture indicates a rather restricted motion determined by the lipids for the charged lidocaine whereas the uncharged molecules are free to diffuse in the lateral directions as well as to jump from one side of the bilayer to the other. Also the positions in the bilayer of lidocaine are different for the two molecules. The charged molecules exhibit a well defined position at the lipid headgroup region with preferential orientation along the bilayer normal and with the

polar N atom directed to the membrane surface. In contrast, the uncharged molecules are located further down in the upper part of the lipid tails with presumable perpendicular to the normal orientation. They assume however orientation parallel to the normal when they diffuse from one side of the bilayer to another. The orientational order of the lidocaine molecules was analyzed employing a simple mean field approach based on the Meier–Saupe theory. The average interaction parameters derived from this analysis confirmed the assumption about stronger interactions between charged lidocaine molecules and the lipid environment. The data obtained are in line with previously made assumptions [13,42] that the neutral form of local anesthetics such as lidocaine is important, due to its higher mobility, for delivering the drug to the interior of membrane, while the charged form being responsible for the anesthetic action.

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References

[1] J.F. Butterworth, G.R. Strichartz, Molecular mechanisms of local anesthesia: a review, Anesthesiology 72 (1990) 711–734.
[2] B. Nilius, K. Benndorf, F. Markwardt, Effects of lidocaine on single cardiac sodium-channels, J. Mol. Cell. Cardiol. 19 (1987).
[3] T. Weiser, Comparison of the effect of four Na^+ channel analgesics on TTX-resistant Na^+ currents in rat sensory neurons recombinant Mav 1.2 channels, Neurosci. Lett. 395 (2006) 179–184.
[4] T. Narahashi, D.T. Frazier, M. Yamada, Cationic forms of local anesthetics block action potentials from inside nerve membrane, Nature 223 (1969) 748–749.
[5] M.F. Sheets, D.A. Hanck, Molecular action of lidocaine on the voltage sensors of sodium channels, J. Gen. Physiol. 121 (2003) 163–175.
[6] G.M. Lipkind, H.A. Fozzard, Molecular modeling of local anesthetic drug binding by voltage-gated sodium channels, Mol. Pharmacol. 68 (2005) 1611–1622.
[7] D. Papahadjoupoulos, K. Jacobson, G. Poste, G. Shepherd, Effect of local anesthetics on membrane properties. 1. Changes in fluidity of phospholipids bilayers, Biochim. Biophys. Acta 394 (1975) 504–519.
[8] Y. Boulanger, S. Schreier, I.C.P. Smith, Molecular details of anesthetic-lipid interaction as seen by deuterium and phosphorus-31 nuclear magnetic resonance, Biochemistry 20 (1981) 6824–6830.
[9] I. Ueda, T. Yoshida, Hydration of lipid membranes and the action mechanisms of anesthetics and alcohols, Chem. Phys. Lipids 101 (1999) 65–79.
[10] H. Matsuki, M. Yamanaka, H. Kamaya, S. Kaneshina, I. Ueda, Dissociation equilibrium between uncharged and charged local anesthetic lidocaine in a surface-adsorbed film, Colloid Polym. Sci. 283 (2005) 512–520.
[11] A. Avdeef, K.J. Box, J.E.A. Comer, C. Hibbert, K.Y. Tam, pH-metric logP10. Determination of liposomal membrane-water partition coefficients of ionizable drugs, Pharm. Res. 15 (1998) 209–215.
[12] I. Ueda, J.S. Chiou, P.R. Krishna, H. Kamaya, Local anesthetics destabilize lipid membranes by breaking hydration shell— infrared and calorimetry studies, Biochim. Biophys. Acta 1190 (1994) 421–429.
[13] L.F. Fraceto, L.M.A. de Pinto, L. Franzoni, A.A.C. Braga, A. Spisni, S. Schreier, E. de Paula, Spectroscopic evidence for a preferential location of lidocaine inside phospholipid bilayers, Biophys. Chem. 99 (2002) 229–243.
[14] L.F. Fraceto, A. Spisni, S. Schreier, E. de Paula, Differential effects of uncharged aminoamide local anesthetics on phospholipid bilayers, as monitored by 1H-NMR measurements, Biophys. Chem. 115 (2005) 11–18.
[15] R.W. Pastor, Computer simulations of lipid bilayers, Curr. Opin. Struct. Biol. 4 (1994) 486–492.
[16] D.P. Tieleman, H.J.C. Berendsen, Molecular dynamics simulations of a fully hydrated dipalmitoylphosphatidylcholine bilayer with different macroscopic boundary conditions and parameters, J. Chem. Phys. 105 (1996) 4871–4880.
[17] O. Berger, O. Edholm, F. Jahnig, Molecular dynamics simulations of a fluid bilayer of dipalmitoylphosphatidylcholine at full hydration, constant pressure and constant temperature, Biophys. J. 72 (1997) 2002–2013.
[18] H.L. Scott, Modeling the lipid component of membranes, Curr. Opin. Struct. Biol. 12 (2002) 495–502.
[19] F.L. Lopes, S.O. Nielsen, M.L. Klein, Hydrogen bonding structure and dynamics of water at the dipalmitoylphosphatidylcholine lipid bilayer surface from a molecular dynamics simulation, J. Phys. Chem., B 108 (2004) 6603–6610.
[20] C.J. Högberg, A.P. Lyubartsev, A molecular dynamics investigation of the influence of hydration and temperature on structural and dynamical properties of a dimyristoylphosphatidylcholine bilayer, J. Phys. Chem., B 110 (2006) 14326–14336.
[21] E. Falck, M. Patra, M. Karttunen, M.T. Hyvönen, I. Vattulainen, Lessons of slicing membranes: interplay of packing, free area, and lateral diffusion in phospholipid/cholesterol bilayers, Biophys. J. 87 (2004) 1076–1091.
[22] 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.
[23] H.J.C. Berendsen, J.P.M Postma, W.F. van Gunsteren, J. Harmans, in: B. Pullman (Ed.), Intermolecular Forces, Reidel, Dordrecht, 1981, pp. 331–342.
[24] A.W. Schuettelkopf, D.M.F. van Aalten, PRODRG— a tool for high-throughput crystallography of protein-ligand complexes, Acta Crystallogr., D Biol. Crystallogr. 60 (2004) 1355–1363.
[25] E. Lindahl, B. Hess, D. van der Spoel, GROMACS 3.0: a package for molecular simulations and trajectory analysis, J. Mol. Model. 7 (2001) 306–317.
[26] B. Hess, H. Bekker, H.J.C. Berendssen, J.G.E. Fraaije, LINCS: a linear constraint solver for molecular simulations, J. Comput. Chem. 18 (1997) 1463–1472.
[27] W.G. Hoover, Constant pressure equations of motion, Phys. Rev., A 34 (1986) 2499–2500.
[28] M. Parinello, A. Rahman, Crystal structure and pair potentials: a molecular-dynamics study, Phys. Rev. Lett. 45 (1980) 1196–1199.
[29] T.A. Darden, D. York, L. Pedersen, Particle mesh Ewald: an *NlogN* method for Ewald sums in large systems, J. Chem. Phys. 98 (1993) 10089–10092.
[30] M. Patra, M. Karttunen, M.T. Hyvönen, E. Falck, P. Lindqvist, I. Vattulainen, Molecular dynamics simulation of lipid bilayers: major artifacts due to truncating of electrostatic interactions, Biophys. J. 84 (2003) 3636–3645.
[31] J. Wohlert, O. Edholm, Electrostatics in lipid membranes: a computer simulation study, Biophys. J. 87 (2004) 2433–2445.
[32] A.H. De Vries, I. Chandrasekhar, W.F. van Gunsteren, P.H. Hunenberger, Molecular dynamics simulations of phospholipid bilayers: influence of artificial periodicity, system size and simulation time, J. Phys. Chem. B 109 (2005) 11643–11652.
[33] A.P. Lyubartsev, S.P. Jacobsson, G. Sundholm, A. Laaksonen, Solubility of organic compounds in water/octanol systems. An expanded ensemble molecular dynamics simulation study of logP parameters, J. Phys. Chem. B 108 (2001) 7775–7782.
[34] P. Atkins, Physical Chemistry, 6-th edition,University Press, Oxford, 1998.
[35] J.W. Emsley, H. Rauzah, G.R. Luckhurst, G.N. Rumbles, F.R. Viloria, The Saupe ordering matrices for solutes in uniaxial liquid crystals, Mol. Phys. 49 (1983) 1321–1335.
[36] R.Y. Dong, Nuclear Magnetic Resonance of Liquid Crystals, Springer, New York, 1994.

[37] P.G. de Gennes, J. Prost, The Physics of Liquid Crystals, Clarendon Press, Oxford, 1993.
[38] R.L. Humphries, P.G. James, G.R. Luckhurst, A molecular field treatment of liquid crystalline mixtures, Symp. Faraday Soc. 5 (1971) 107–118.
[39] W.E. Palke, J.M. Emsley, D.J. Tildesley, Computer simulations of the behaviour of a solute in a model liquid crystalline solvent, Mol. Phys. 82 (1994) 177–192.
[40] D. Sandström, A.V. Komolkin, A. Maliniak, Orientational order in a liquid crystalline mixture studied by molecular dynamics simulation and NMR, J. Chem. Phys. 104 (1996) 9620–9628.
[41] D. Sandström, A.V. Komolkin, A. Maliniak, Molecular dynamics simulation of a liquid crystalline mixture, J. Chem. Phys. 106 (1997) 7438–7447.
[42] J.D. da M. Neto, R.B. de Alencastro, Theoretical studies on local anesthetics: procaine, lidocaine, tetracaine, bupivacaine, and dibucaine— neutral and monoprotonated, Int. J. Quant. Chem. 61 (1997) 959–980.