How lidocaine influence the bilayer thickness and bending elasticity of biomembranes

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Abstract. We have studied how local anesthetics influence the structural and dynamical properties of model bio-membranes. The measurements of small-angle neutron scattering have been performed on 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) unilamellar vesicles with different concentrations of lidocaine in D2O to determine the bilayer thickness as a function of the lidocaine concentration. The neutron-spin echo spectroscopy (NSE) has been used to study the influence of lidocaine on the bending elasticity of DMPC bilayers in fluid crystal phase (Lα) and the ripple gel (Pβ') phase.

1. Introduction
Local anesthetics (LAs) are known to produce loss of sensation to pain in the certain area of the body without the loss of consciousness [1]. However, the mechanism of their action is still unclear. The current consensus is that the site of action of LAs is within the cellular membrane of the neuron: LAs produce a blockade of nerve impulse by blocking Na+ voltage-gated ion channels of nerve membranes and slowing the rate of depolarization [2-4]. In order to block the sodium channel the molecules of LAs directly bind to the membrane transport proteins affecting their function [3-6]. Moreover, recent experiments by a number of methods [7-9] have demonstrated that LAs also interact with the lipid membranes and alter the organizational properties of membranes. Such changes on biological membranes could interfere with lipid-protein interactions, and lead to protein conformational changes with a reflection on their activity. In our study, unilamellar vesicles (ULV) of DMPC, one of the most extensively studied ester-linked phospholipids with regard to structural and thermodynamic properties, mixed with different concentrations of lidocaine, the most clinically used LAs, were studied. Differential scanning calorimetry (DSC) has been used to investigate the effect of lidocaine on the phase behaviour of DMPC bilayers in deuterated water solutions. We have used small angle neutron scattering (SANS) to investigate the influence of LAs on the bilayer thickness of DMPC bilayers in aqueous solutions. Furthermore, the bending elasticities (κc) of DMPC ULV bilayers with different concentrations of lidocaine in different phases were measured by Neutron Spin Echo (NSE) technique. The intermediate scattering function acquired through NSE was explained by the Zilman-Granek model [10, 11] for 2-D membranes.
2. Materials and methods

Synthetic 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) was purchased from Avanti® Polar Lipids, (Alabaster, AL) and used without further purification. 2-diethylamino-N-(2,6-dimethylphenyl)acetamide (lidocaine) hydrochloride monohydrate was purchased from Sigma Chemicals (St. Louis, MO) in the solid form. D₂O (99 % atomic percent) was purchased from Cambridge Isotope Labs, (Andover, MA). DMPC Bilayers of unilamellar vesicles were prepared by extrusion method using a polycarbonate filter with pore diameter of 1000 Å [12]. A lipid concentration of a mass fraction of 1.0 % in D₂O was used for SANS, 2.0 % for NSE and 10.0% for DSC measurement. The averaged radii of the vesicles in 30 ºC, which are about 450 Å, were characterized by dynamic light scattering (DLS).

DSC was used to investigate the effect of LAs on the phase-transition temperatures of DMPC bilayer membrane. The thermotropic phase transition of multilamellar vesicles of molar ratio \( R \) of lidocaine/DMPC = 0, 0.2, 0.5, 0.7, 1.0 and 1.5 (lipid concentration in D₂O of a mass fraction of 10 %) was determined by TA instruments Q1000 differential scanning calorimeter (New castle, DE). The temperature scanning was from 30 ºC to 0 ºC, then returning back 30 ºC with the temperature accuracy of ± 0.1 ºC. The heating and cooling scanning rates were 1 ºC per min.

SANS measurements were performed at NG3 30m SANS instrument located at NIST Center for Neutron Research (NCNR) [13]. Neutrons of wavelength \( \lambda = 8.4 \) Å with a wavelength spread of \( \Delta \lambda / \lambda = 11 \) % were used. Data were collected with a two-dimensional detector at three different sample-to-detector distances (1.3m, 4.0m and 13.7m) in order to span the range of scattering vectors \( q \) from \( 2 \times 10^{-3} \) to \( 4 \times 10^{-1} \) Å⁻¹. Samples were contained in standard, 1-mm-path-length quartz cells. The data were corrected for instrumental and empty cell backgrounds using the data reduction macros of Igor Pro supplied by NIST [15]. Samples of \( R = 0 \), 0.2, 0.5, 2.0 and 5.0 were measured at 30 ºC in \( \lambda \) phase, above the main transition temperature of DMPC. The temperature was controlled by the water circulation with an accuracy of ± 0.1 °C. The measured scattering intensity of DMPC ULV bilayers were fitted as polydispersed spherical vesicles by single-strip model [14-16].

NSE spectrometer measures in the time domain, the real part of the intermediate scattering function, \( I(q,t) \). In Zilman-Granek model for 2-D membranes, the time decay of \( I(q,t) \) originating from thermal undulations of isolated single membranes can be predicted to exhibit stretched exponential decay:

\[
I(q,t) = I(q,0) e^{-(\Gamma t)^{\beta/2}}
\]

where \( \Gamma \) is the relaxation rate, and related to the bending elasticity as:

\[
\Gamma = 0.025 \gamma_k \left( \frac{k_B T}{k_c} \right)^{1/2} \frac{k_B T}{\eta} q^3
\]

where \( \eta \) is a viscosity of a solvent, and a factor \( \gamma_k \) originates from averaging over the angle between the wave vector and the plaquette surface normal in the calculation of \( I(q, t)/I(q, 0) \). The parameter \( \gamma_k \) unifies when \( k_c >> k_BT \). We use three times the value of average solvent (\( \eta = 3 \eta_{\text{solvent}} \)) for viscosity \( \eta \) when taking the local dissipation at the membrane into consideration [17-19]. Our data were taken on the spectrometer located on the NG5 guide of NCNR. Neutrons with wavelengths of 8 Å and 11.6 Å were used. The range of momentum transfer \( q \) was from 0.03 Å⁻¹ to 0.12 Å⁻¹. The reduced NSE data were analyzed using NCNR program DAVE. The temperature dependence of the bending elasticity was measured for samples with molar ratio \( R \) of Lidocaine and DMPC = 0, 0.5 and 2.0 at several temperatures from 10 °C to 30 °C.

3. Results and discussion

DSC measurement shows that the presence of lidocaine shifts the main transition temperature \( (T_m) \) of DMPC bilayers and depresses and broadens its endothermic peak. \( T_m \) of DMPC bilayers (red triangles) as a function of \( R \) was plotted in Figure 1. These scans show that the presence of lidocaine progressively depresses the main transition temperature and enthalpy in an approximately quadratic...
dependence. At the highest \( R = 1.5 \), the concentration of lidocaine in \( D_2O \) went over to about 200 mmol/kg and the main transition temperature was depressed to as low as 18.8 °C. This anesthetic-induced depression of main transition temperature of DMPC bilayers is an evidence of the change in membrane organization by LAs. It is necessary to investigate the structural change of DMPC model bio-membranes by the presence of LAs.

From SANS result, Bilayer thicknesses \( d \) of DMPC bilayers in various \( R \) were calculated and the dependence of \( d \) (black circles) on \( R \) is also plotted in Figure 1. Bilayer thickness of vesicles \( d \) decreases with \( R \) in quadratic dependence. The decrease is in agreement with the conclusions of the previous NMR study of phosphatidylcholines-tetracaine hydrochloride interaction.

LAs molecules penetrate into the lipid bilayers: the hydrophilic parts of LAs mostly remain in phospholipid head-group level, and their lipophilic parts insert little in the bilayer hydrophobic region [20]. The depth of the insertion depends on polarity of lipid head groups and pH [21, 22]. The molecules of LAs with shorter length (lidocaine length \( \approx 9.4 \text{Å} \) loose the packing of the lipids with longer length (DMPC length \( \approx 25\text{Å} \)), and induce the lateral membrane expansion. The intercalation of LAs provides more inter-lipid spaces for lipid hydrophobic chains, and then results in the decrease of bilayer thickness. Moreover, lateral membrane expansion provides hydrophobic tails more space to swing, and then disorders the hydrophobic parts of bilayers. This disordering in hydrophobic parts causes the decrease of the main transition temperature.

### Table 1. Bending elasticity \( \kappa_c/K_BT \) of DMPC bilayers with different \( R \) in \( D_2O \)

| \( R \) | 10 | 15 | 20 | 23 | 25 | 30 |
|---|---|---|---|---|---|---|
| 0  | 151.0±6.10 | 120.7±7.25 | 20.4±0.68 | 16.7±0.48 |
| 0.5 | 119.5±7.67 | 68.2±3.34 | 31.8±1.17 | 35.9±1.23 |
| 2.0 | 114.4±10.46 | 69.4±4.70 | 31.0±1.40 | 33.8±1.46 |

In our previous research [19] we have examined the effect of temperature on bending elasticity \( \kappa_c \) of pure lipid ULVs in \( D_2O \): At \( T >> T_m \), \( \kappa_c \) of pure DMPC bilayers is independent of temperature, and the temperature has a minimal effect on the properties of the lipid bilayer in \( L_α \) phase; when the temperature is approaching the vicinity of the main transition temperature, \( \kappa_c \) become slightly larger [23]; When temperature goes across the main transition temperature and into \( Pβ' \) phase, the \( \kappa_c \) rapidly increases to 6-10 times of \( \kappa_c \) in \( L_α \) phase. In present study, bending elasticities of DMPC bilayer with \( R=0, 0.5 \) and 2.0 were calculated by equation (2) and shown in Table 1. In the vicinity of the main transition temperature of DMPC bilayers (data at 25 and 30 °C), \( \kappa_c \) fluctuates from 16.7\( k_BT \) for \( R=0 \) to 33.8 \( k_BT \) for \( R=2.0 \). The effect of lidocaine on \( \kappa_c \) in \( L_α \) phase isn’t as obvious as its effect on transition temperature. In the discussion of our SANS result, we confirmed that the intercalation of lidocaine causes more inter-lipid spaces for lipid hydrophobic chains, so that the tail-tail interaction decreased.
However, the interaction of headgroups increased because of the possible closer packing in hydrophilic parts of DMPC and lidocaine. Under the dual effects of hydrophobic and hydrophilic groups, $\kappa_c$ change of DMPC ULVs with lidocaine in $L_\alpha$ phase is negligible. At $T_m=23$ °C, $\kappa_c$ of sample of $R=0$ was 6 times of its $\kappa_c$ in $L_\alpha$ phase, however for samples of $R=0.5$, and 2.0, $\kappa_c$ were only 1-3 times of their $\kappa_c$ in $L_\alpha$ phase. The results of NSE also confirmed the decrease of $T_m$ by lidocaine. The presence of lidocaine disordered the arrangement of hydrophobic parts of phospholipid bilayers, thus prevent "gelling" at lower temperatures.

4. Conclusions

We have used DSC, SANS and NSE to measure the influence of lidocaine on the structural and dynamic properties of DMPC phospholipid bilayers. Our results examined that the molecules of LAs loose the packing of the lipids, and induce the lateral membrane expansion. The intercalation of LAs molecules provides more inter-lipid spaces for lipid hydrophobic chains, and finally results in the decrease of bilayer thickness, decrease of the main transition temperature from $L_\alpha$ to $P_{\beta}$' phase and doubled bending elasticity in $L_\alpha$ phase. It is suggested that the increase of the head area and the bending elasticity makes the membrane-protein interaction larger, and possibly the appearance of the blockage of ion channels.

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References

[1] Braun H 1914 Local Anesthesia: Its Scientific Basis and Practical Use
[2] Lee A G 1976 Nature 262 545-548
[3] S.Wang, C. Nau, G.K. Wang, 2000 Biophys. J. 79 1379-1387.
[4] M.F. Sheets, D. A. Hanck, 2003 J. Gen. Physiol. 121 163-175.
[5] C. Nau, G.K. Wang, 2004 J. membrane Biol. 201 1-8.
[6] M. Gregory, H.A. Fozzard, 2005 Mol. Pharmacol. 68 1611-1622.
[7] Earnest J P, Limbacher H P, Mclnamie M G et al. 1986 Biophys. J. 49 A594
[8] Kuroda Y, Nasu H, Fujiwara Y et al. 2000 J. of membrane Biol. 177 117-128
[9] Uhrikova D, Rapp G, Yaradaikin S, et al. 2004 Biochemistry 109 361-373
[10] Zilman A and Granek R 1996 Phys. Rev. Lett. 77 4788
[11] Zilman A and Granek R 2002 Chem. Phys. 284 195
[12] Nayar R, Hope M J and Cullis P R 1989 Biochim. Biophys. Acta 986 200-206
[13] Glinka C J, et al. 1998 J. Appl. Cryst. 31 430
[14] Kline S R 2006 J. Appl. Cryst. 39 6 895
[15] Kiselev M, Zemlyanaya E, Aswal V and neubert R 2006 Eur. Biophys J. 35 477-493
[16] Kucerkla N, Nagle J, Feller S and Balgavy P 2004 Phys. Rev. E 69 051903
[17] Farrago B et al. 1995 Physica B 213 712
[18] Takeda T et al. 1999 J. Phys. Chem. Solids 60 1375
[19] Yi Z, Bossev D P and Nagao M 2009 J. of Phys.: Condens. Matter 21 155104
[20] Smith I, Auger M and jarrell H 1991 Annals of the New York of Academy of Sci. 625 668-684
[21] Boulanger Y, Schreier S and Smith I C P 1981 Biochemistry 20 6824-6830
[22] Hogberg C, maliniak A and Lyubartsev A 2007 Biophys. Chem. 125 416-424
[23] Seto H, Yamada NL., Nagao M, Hishida M. and Takeda T. 2008 Euro. Phys. J. E. 26 217