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Short Communication

Study of interaction of long-chain *n*-alcohols with fluid DOPC bilayers by a lateral pressure sensitive fluorescence probe

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Abstract. The excimer 1,2-dipyrenedecanoyl-sn-glycero-3-phosphatidylcholine ($dipy_{10}PC$) fluorescence probe was used to determine effects of aliphatic alcohols ($C_nH_{2n+1}OH$, n = 12–18 is the even number of carbons in alkyl chain) on fluid dioleoylphosphatidylcholine (DOPC) + dioleoylphosphatidylserine (DOPS) bilayers in multilamellar vesicles at molar ratio DOPC/DOPS = 24.7. The excimer to monomer fluorescence intensity ratio increases with the increase of $C_nH_{2n+1}OH$/DOPC molar ratio and decreases with the $C_nH_{2n+1}OH$ alkyl chain length n at a constant $C_nH_{2n+1}OH$/DOPC = 0.4 molar ratio. These effects indicate changes in the bilayer lateral pressure on the level of pyrenyl moieties location.

Key words: Lipid bilayer — Dioleoylphosphatidylcholine — Alkan-1-ol — Dipyrenylphosphatidylcholine

Long-chain primary aliphatic alcohols (alkan-1-ol, abbreviation $C_nH_{2n+1}OH$; n is the number of carbons in the aliphatic chain) partition into lipid bilayers of biomembranes and change their structural and dynamical properties. Structural changes of fluid bilayers in multilamellar and unilamellar dioleoylphosphatidylcholine (DOPC) vesicles were studied recently by small-angle neutron diffraction and scattering methods (Petrenko et al. 2010; Klacsová et al. 2011). It was found that the bilayer thickness decreases with $C_nH_{2n+1}OH$/DOPC molar ratio and increases with $C_nH_{2n+1}OH$ chain length n at a constant $C_nH_{2n+1}OH$/DOPC molar ratio; simultaneously, the bilayer expands laterally with the partial molar $C_nH_{2n+1}OH$ interface area increasing with the chain length n. All these structural modifications induced by studied $C_nH_{2n+1}OH$s were reproduced in molecular dynamics (MD) simulations (Klacsová et al. 2011). MD simulations predicted also changes in the intrabilayer lateral pressure profile (Griepernau and Bockmann 2008). It is well known that $C_nH_{2n+1}OH$s are general anesthetics with a maximum potency for $C_{12}H_{25}OH$ (Pringle et al. 1981). It was hypothesized that their effects on postsynaptic ligand-gated ion channels involved in anesthesia could be caused by shifts in the distribution of lateral pressure within bilayer (Cantor 1997, 2001). In the present work, we study changes in the bilayer lateral pressure using the 1,2-dipyrenedecanoyl-sn-glycero-3-phosphatidylcholine ($dipy_{10}PC$) fluorescence probe. The $dipy_{10}PC$ probe has pyrenyl moieties attached to the ends of equal length acyl chains on a phosphatidylcholine molecule and its ultraviolet excitation results in both monomer and excimer pyrenyl fluorescence in phosphatidylcholine bilayers (Sunamoto et al. 1980). According to Templer et al. (1998), the ratio of intramolecular excimer to monomer fluorescence intensity can be used as a measure of the lateral pressure.

In previous experiments (Klacsová et al. 2011), the small amount (4 wt%) of dioleoylphosphatidylserine (DOPS) was added to DOPC to charge the bilayer surface negatively and thus to prevent vesicle aggregation after preparation. In present work we added the 4 wt% DOPS to DOPC as well. DOPC and DOPS were purchased from Avanti Polar Lipids (Alabaster, USA), $dipy_{10}PC$ was obtained from Invitrogen

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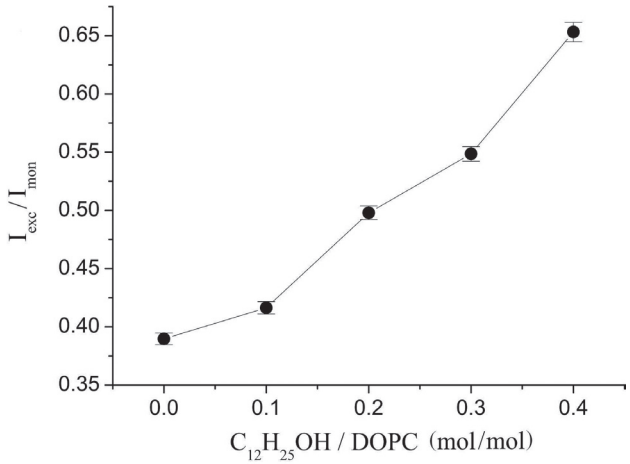

Figure 1. Dependence of the I_{exc}/I_{mon} intensity ratio on the $C_{12}H_{25}OH$/DOPC molar ratio at 20°C. I_{exc} and I_{mon} are emission intensities at 375.5 and 475 nm for dipy$_n$PC monomer and excimer, respectively.

Corporation (Carlsbad, USA) and $C_nH_{2n+1}OH$s (with n = 12, 14, 16, 18) from Sigma (St. Luis, USA). The organic solvents of spectral purity were obtained from Slavus (Bratislava, Slovakia). Lipids (DOPC and DOPS), dipy$_{10}$PC and $C_nH_{2n+1}OH$ were dissolved at known concentrations in an organic solvent (chloroform:methanol = 1:1 v/v) and stored at –20°C. Required volumes of dipy$_{10}$PC and $C_nH_{2n+1}OH$ solutions were added to solutions of lipids. The organic solvent was evaporated under a stream of nitrogen gas and its rest removed under vacuum. Dried samples were hydrated by the addition of deionized water (Milli-Q water with resistivity 18.2 MΩ·cm) and homogenized by vigorous vortex mixing. Prepared dispersions were sonicated during several seconds and stored in a refrigerator for 20 hours for equilibration. After that the samples were again vortexed and sonicated. The final concentration of a lipid or lipid+$C_nH_{2n+1}OH$ in the sample was 0.127 mM and the dipy$_{10}$PC:(DOPC+DOPS+$C_nH_{2n+1}OH$) molar ratio was 1:1500. At this low molar concentrations of dipy$_n$PC the fluorescence from intramolecular excimers predominates over intermolecular one (Vauhkonen et al. 1990). Fluorescence measurements were performed using the 10 mm quartz cuvette in the fluorimeter FluoroMax-4 (HORIBA Jobin Yvon, France). The excitation was at 345 nm and the emission spectrum was collected between 360 and 650 nm with the increment 0.5 nm and integration time for each wavelength 0.1 s. The excitation and emission bandwidths were 3 and 1 nm, respectively. During measurements, the samples were held in a cell which temperature was regulated to within ±0.01°C by a Peltier thermocouple drive. The average relative error of emission intensity was 0.9%. The ratio I_{exc}/I_{mon} was calculated for emission intensities at 375.5 and 475 nm for monomer and excimer emission correspondingly.

With the increase of CnOH/DOPC molar ratio in bilayers, the excimer to monomer fluorescence intensity ratio I_{exc}/I_{mon} increases at a constant temperature. This effect is illustrated in Figure 1 for the $C_{12}H_{25}OH$ homolog in bilayers. It is seen, that I_{exc}/I_{mon} increases which indicates an increase in the bilayer lateral pressure on the level of pyrenyl moieties of dipy$_{10}$PC fluorescence probe location in the bilayer – the lateral pressure forces the pyrenyl moieties closer to each other, which increases the probability of excimer formation (see Templer et al. 1998). Figure 2 demonstrates the effect of $C_nH_{2n+1}OH$ on the fluorescence intensity ratio I_{exc}/I_{mon} at a fixed temperature and constant molar ratio $C_nH_{2n+1}OH$/DOPC = 0.4. The maximum effect on the lateral pressure is seen for $C_{12}H_{25}OH$ homolog; the influence of alcohols diminishes with the increase of $C_nH_{2n+1}OH$ alkyl chain length n and the measured I_{exc}/I_{mon} values approach the control values for $C_{18}H_{37}OH$. This finding is in accord with recent bilayer thickness measurements of Petrenko et al. (2010) and Klacsová et al. (2011) – as the alkyl length of $C_nH_{2n+1}OH$ approaches the length of DOPC acyl chain lengths, the bilayer thickness is nearly the same as in the control DOPC bilayers without $C_nH_{2n+1}OH$.

In conclusion, the alcohol and lipid hydrophobic chain length mismatch seems to be a critical factor for both the physical (bilayer thickness decrease, lateral pressure increase) and anesthetic effects of $C_nH_{2n+1}OH$s , which are maximal for $C_{12}H_{25}OH$ and which disappear with the increase of $C_nH_{2n+1}OH$ alkyl chain length.

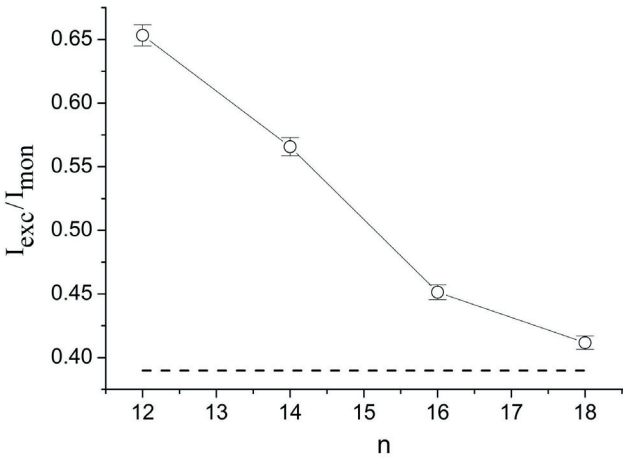

Figure 2. Dependence of the I_{exc}/I_{mon} ratio on the $C_nH_{2n+1}OH$ chain length n at molar ratio $C_nH_{2n+1}OH$/DOPC = 0.4. Temperature 20°C. I_{exc} and I_{mon} are emission intensities at 375.5 and 475 nm for dipy$_n$PC monomer and excimer, respectively. The dashed line corresponds to DOPC bilayers without $C_nH_{2n+1}OH$.

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