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Properties of Mixed Cationic Membranes studied by Fluorescence Solvent Relaxation

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Abstract The need for detailed biophysical description of cationic lipid membranes, which are commonly used as gene transfection vectors, led us to study the properties of mixed cationic/zwitterionic lipid bilayers. Fluorescence solvent relaxation measurements of 6-dodecanoyl-2-dimethylaminonaphthalene (Laurdan) incorporated in a membrane consisting of cationic dimyristoyltrimethylammoniumpropane (DMTAP) and zwitterionic dimyristoylphosphatidylcholine (DMPC) were performed. The obtained results are compared with a recently measured system consisting of dioleoyltrimethylammoniumpropane (DOTAP) and dioleoylphosphatidylcholine (DOPC) (Jurkiewicz et al. Langmuir 22:8741–8749, 2006). The similar nonmonotonic dependence of the relaxation kinetics on cationic lipid content in the membrane was present for both systems. While the slowest solvent relaxation have been observed for 30 mol% of DOTAP in the DOPC bilayer (Jurkiewicz et al. Langmuir 22:8741–8749, 2006), for DMPC/DMTAP system it was found at 45 mol% of DMTAP, which agrees with the literature. Both membranes increased their hydration upon increased cationic lipid content.

Keywords Time-resolved emission spectroscopy · Solvent relaxation · Membrane interface · Cationic lipids · Lipid bilayer hydration

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Introduction

Cationic lipids are often used in gene delivery protocols [2]. They interact with negatively charged nucleic acids serving as condensers [3], protecting genetic material from degradation, and enhancing transfection efficiency [2]. One of the best known examples is dioleoyltrimethylammonium-propane (DOTAP), which is a synthetic phospholipid with an electric charge +1 on its trimethylammonium-propane (TAP) headgroup [4]. Cationic lipids are often mixed with a neutral, so-called, helper lipid (e.g. lecithin or ethanolamine) [5]. Since the neutral lipids are usually zwitterions, the resulting mixtures posses interesting physical properties, i.e. their phase diagrams exhibit pronounced maxima, which reveals strongly nonideal mixing [6]. This phase behavior was attributed to the rotation of the zwitterionic headgroup (i.e., P-N vector) caused by its electrostatic interaction with the cationic headgroup (i.e., repulsion of N^+ and attraction of P^-) [7]. This idea came from nuclear magnetic resonance (NMR) measurements [7, 8]. However, the hydration of the NMR lipid samples differs from the one of free standing bilayers.

Contrary to NMR, fluorescence solvent relaxation technique (SR), used in the present work, is capable of monitoring both hydration and mobility (packing) of the lipid bilayer under physiologically relevant conditions (fully hydrated liquid crystalline membrane) [9–11].

In a recent work we have shown that the P-N dipole orientation of the dioleoylphosphatidylcholine (DOPC) molecules strongly affects mobility in the headgroup region of DOTAP/DOPC bilayers [1]. While the membrane hydration is affected only moderately by the presence of DOTAP, the mean relaxation time (inversely proportional to the mobility) plotted versus DOTAP content showed a distinct maximum at 30 mol% of DOTAP. We attribute this

non-monotonic dependence to the straightening of the P-N vector of DOPC upon electrostatic interaction with DOTAP. Position of this maximum differs from the one obtained recently in molecular dynamics simulations [12] and theoretical studies [13], where the minimum area per lipid have been found for 50 mol% of cationic lipid. Both works, however, concerned dimyristoylphosphatidylcholine (DMPC)/dimyristoyltrimethylammoniumpropane (DMTAP) lipids, which possess myristoyl, instead of oleoyl, backbones. This motivated the SR study on DMPC/DMTAP bilayers. The present paper serves to compare the two systems (DOPC/DOTAP and DMPC/DMTAP) and to summarize our SR studies on the hydration and mobility of cationic membranes.

Experimental

1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and 1,2-dimyristoyl-3-trimethylammonium-propane (DMTAP) were obtained from Avanti Polar Lipids, Inc. (Alabaster, AL, USA). A fluorescence probe, 6-dodecanoyl-2-dimethylaminonaphthalene (Laurdan) was purchased from Molecular Probes (Eugene, OR, USA). 4-2-hydroxyethyl-1-piperazineethanesulfonic acid (HEPES) was supplied by Fluka (Buchs, Switzerland) and all solvents of spectroscopic grade by Merck (Darmstadt, Germany). Extruded 100 nm liposomes in 10 mM HEPES (pH=7.4, 100 mM NaCl) were prepared as previously described [1].

All steady-state fluorescence measurements were performed on a Fluorolog-3 spectrofluorometer (model FL3–11; Jobin Yvon Inc., Edison, NJ, USA) equipped with a xenon-arc lamp. Temperature was maintained within ±0.1 °C using a water-circulating bath. Fluorescence decays were recorded on a 5,000 U Single Photon Counting setup equipped with 370 nm NanoLED diode (IBH, Glasgow, UK) and a cooled Hamamatsu R3809U-50 microchannel plate. The decays were recorded at a series of emission wavelengths spanning the steady-state spectrum (400–540 nm) in 10 nm steps at magic angle polarization. Data collected in 8,192 channels (0.014 ns per channel) was fitted with multi-exponential functions (2 or 3 exponential components were used) using the iterative reconvolution procedure (IBH DAS6 software). The time resolution of our instrumentation was about 20 ps.

On the basis of previous experience [1] we have chosen Laurdan as the most appropriate for this study.

Fluorescence solvent relaxation

For a detailed description of the method please refer to our previous papers [1, 9, 10, 14]. In short, time-resolved emission spectra (TRES) obtained by the spectral reconstruction method, were fitted to a log-normal functions in order to determine their position ν(t) and their width (full width at half-maximum). The correlation function:

$$C(t) = \frac{\nu(t) - \nu(\infty)}{\nu(0) - \nu(\infty)} = \frac{\nu(t) - \nu(\infty)}{\Delta\nu}, \quad (1)$$

was calculated by taking the estimated time-zero spectrum as ν(0) [9]. To quantify the solvent relaxation process, two parameters are determined. The first one, Δν, represents the overall emission shift, which is proportional to the polarity of the dye environment. Since in a phospholipid bilayer polarity is mainly determined by water molecules, therefore, Δν reflects the extent of membrane hydration. The second parameter, relaxation time, describes the mobility of the solvent molecules. In the phospholipid bilayer at the level of glycerol, where the dye is located, water hydrating the membrane is fully bound to the phospholipid molecules. Therefore, the relaxation kinetics observed in membranes is attributed to the collective relaxation of the dye environment and reflects membrane dynamics rather than the motions of water molecules alone. The solvent relaxation time was calculated as:

$$\tau \equiv \int_0^\infty C(t)dt \quad (2)$$

The intrinsic uncertainty for this parameter was assumed to be ~20 ps, based on the time resolution of the experimental setup.

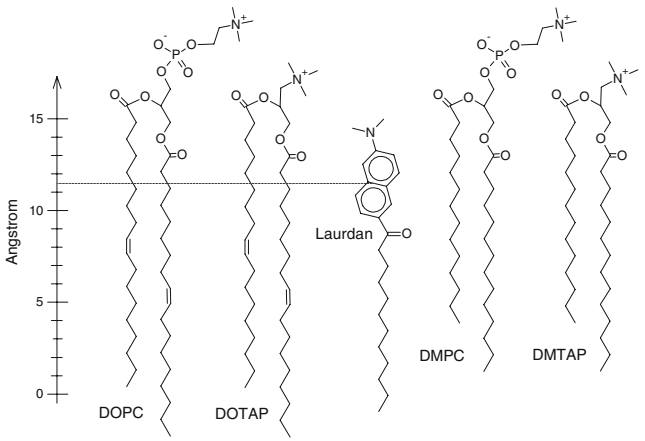

Fig. 1 Structures of the lipid molecules: dioleoylphosphatidylcholine (*DOPC*), dioleoyltrimethylammoniumpropane (*DOTAP*), dimyristoylphosphatidylcholine (*DMPC*), dimyristoyltrimethylammoniumpropane (*DMTAP*); and fluorescent probe 6-dodecanoyl-2-dimethylaminonaphthalene (*Laurdan*). The average position of the molecule of Laurdan is depicted, as evaluated using parallax quenching method for the DOPC membrane [1]

Results and discussion

We measured the influence of cationic lipid DMTAP on the dynamics and hydration of phospholipid bilayer above the phase transition. The structures of lipid molecules (DMPC, DOPC, DMTAP, DOTAP) and fluorescent probe (Laurdan) used in this study are presented in Fig. 1. The content of positively charged compound varied within the range of 0–100 mol%. In Fig. 2 comparison of solvent relaxation $\Delta\nu$ parameter for Laurdan in DMPC/DMTAP LUVs and DOPC/DOTAP LUVs measured previously [1] as a function of cationic lipid content is shown. When analyzing bilayer hydration, which is proportional to the overall spectral shift, $\Delta\nu$, it is important to consider uncertainty of $\Delta\nu$, which for relative evaluations is ~50 cm^{-1}. For both discussed systems a small increase in bilayer hydration was observed for rising percentage of cationic TAP (Fig. 2). For DOPC/DOTAP this increase is very small when probed by Laurdan, however, we showed that for slightly deeper located dye, Patman, increased membrane penetration by water becomes evident [1]. We believe that at Laurdan location DOPC bilayer is already fully hydrated and thus increased water penetration can be observed only for deeper located probes. Seemingly it is different for less hydrated DMPC (lower $\Delta\nu$ values), for which $\Delta\nu$ increase with increasing TAP content is more pronounced. Although, the overall change in DMPC/DMTAP hydration is not large, the bilayer hydrates gradually when percentage of DMTAP increases. The higher hydration of DOPC than DMPC is not a surprise as the double unsaturated backbones of DOPC create molecule with bulky hydrophobic part, hence, the larger area per lipid leaves more space for water at the headgroup region; in comparison with DMPC with saturated myristoyl chains.

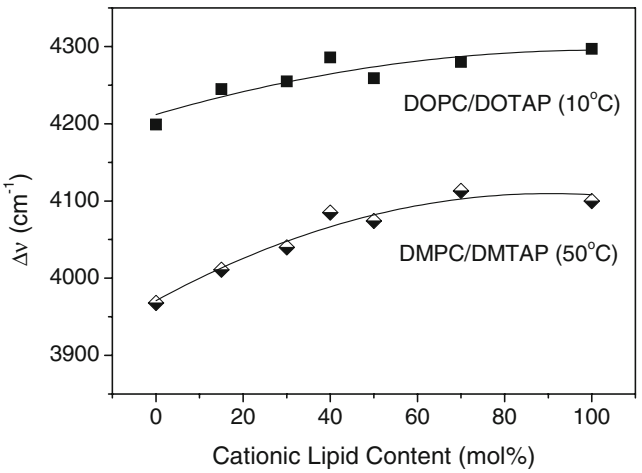

Fig. 2 Solvent relaxation $\Delta\nu$ parameter for Laurdan in DOPC/DOTAP LUVs (*squares*) and DMPC/DMTAP LUVs (*diamonds*) as a function of cationic lipid content. Measurements were performed at 10 °C and 50 °C for DOPC/DOTAP and DMPC/DMTAP systems, respectively. The results for DOPC/DOTAP are taken from [1]. The best polynomial fits (*solid lines*) are shown for eye guidance

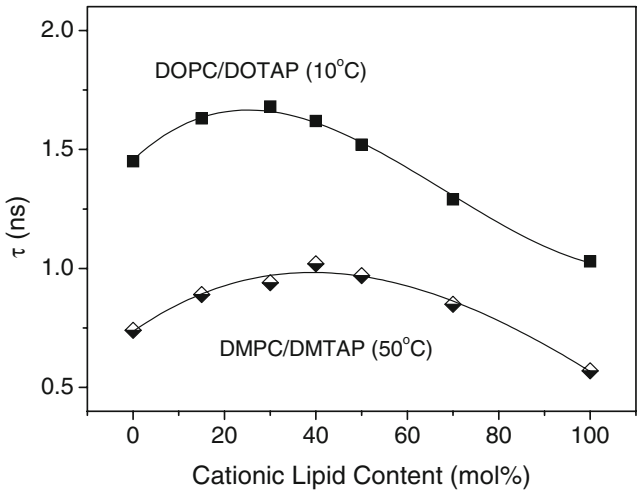

Fig. 3 Mean solvent relaxation time τ (defined by Eq. 2.) for Laurdan in DOPC/DOTAP LUVs (*squares*) and DMPC/DMTAP LUVs (*diamonds*) as a function of cationic lipid content. Measurements were performed at 10 °C and 50 °C for DOPC/DOTAP and DMPC/DMTAP bilayers, respectively. The results for DOPC/DOTAP are taken from [1]. The best polynomial fits (*solid lines*) are shown for eye guidance

The dependences of the relaxation time (calculated according to Eq. 2) on the TAP content in both compared systems are shown in Fig. 3. The relaxation time gives us a measure of membrane lipid mobility (or bilayer rigidity). Maxima of mean relaxation time τ are present for both formulations. The initial increase in relaxation time (Fig. 3 from 0 to 30/45 mol% of TAP) reflects lateral compression of the membrane due to rising of lipid heads. The P-N vector, which in pure PC bilayer is positioned almost parallel to membrane surface, straightens to create angle of ~30° with membrane normal [12], which leads to decrease in cross section area of the PC head and subsequently to membrane compression. Further increase of TAP content in the bilayer does not change P-N vector orientation any more. Instead the repulsion between positively charged TAP groups increases sufficiently to dominate over the forces that compressed the bilayer, which starts to expand. The pure TAP membranes (in the case of both systems) are already less packed than pure PC ones. For DOPC/DOTAP bilayer the maximum relaxation time was observed at ~30 mol% of DOTAP in the mixture [1]. In the case of DMPC/DMTAP this maximum is shifted to approximately 45 mol% of DMTAP. This result is already in very good agreement with the simulations performed by Gurtovenko et al. [12], where the minimum of area per lipid was found at around 50 mol%. The difference in the behavior of DOPC/DOTAP and DMPC/DMTAP systems can be explained by the geometry of these lipids. The DOPC molecule is cylindrical in shape with the headgroup of similar diameter as the one of hydrophobic backbone. The

DMPC has the same head, but its hydrophobic part is thinner (smaller diameter). As a result the membrane area per DMPC molecule is smaller and determined rather by the headgroup than the backbone. That is why the initial compression of DOPC/DOTAP bilayer finishes sooner (at 30 mol% of DOTAP) than for DMPC/DMTAP, since the compressibility of DOPC/DOTAP membrane is in lager extend restricted by its bigger hydrophobic backbone. In the case of DMPC/DMTAP the maximum is broader and strongly pronounced. A comparison between the relaxation kinetics between pure DOPC and DMPC is not as simple as in the case of their hydration. Relaxation time strongly depends on temperature and thus the similar relative temperatures (i.e. relative to the main phase transition temperatures) are often used. However, as we have recently shown, this does not guarantee results that could be directly compared due to the fact that the relaxation kinetics depends on many different factors including probe location, bilayer composition and absolute temperature [15].

Conclusions

Solvent relaxation applied to lipid membranes can provide unique information on their hydration and dynamics, being exceptionally sensitive to the changes occurring within the headgroup region of the lipid bilayer. The characteristics of the cationic/zwitterionic–TAP/PC obtained in this work gives experimental evidence of the headgroup dipoles rearrangement upon the introduction of the positively charged TAP groups in fully hydrated free-standing bilayers. This process results in membrane compression for the moderate TAP content and subsequent expansion for higher TAP concentrations. The maximum of membrane compression is different for DOPC/DOTAP and DMPC/DMTAP, with the later (at 45 mol%) being in very good agreement with molecular dynamics simulations [12]. We found that the studied cationic bilayers become slightly more hydrated with increasing surface charge. These basic studies could be potentially used for screening and control in the field of gene therapy and targeted drug delivery systems.

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