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Phase transition, ordering and lateral diffusion in phospholipid bilayers in the presence of poly(ethylene oxide)

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The thermal behaviour, molecular orientation and lateral diffusion in the bilayered systems of dimyristoylphosphatidylcholine (DMPC) in the presence of poly(ethylene oxide) (PEO) were studied by NMR and DSC techniques, and it was found that PEO decreases the melting temperature (of vesicles and flat multibilayers) and affects the degree of orientation of DMPC molecules relative to the bilayer normal, but it does not influence the lateral diffusion of DMPC molecules.

Poly(ethylene oxides) (PEOs) are biomembrane-active agents frequently used in pharmacy, medicine and molecular biology.[1,2] PEOs are highly soluble in water, and they have low toxicity and low immunogenicity. Therefore, the mechanisms by which PEO molecules interact with phospholipids is of interest. In this work, we studied alterations in phospholipid bilayer properties, such as the phase state and melting behaviour of the lipid system, lipid molecule orientation and lateral diffusion in the membrane that were induced by the PEO–lipid interaction.

Commercial phospholipid dimyristoylphosphatidylcholine (DMPC) from Avanti Polar Lipids and PEO (MW 6000) from Sigma-Aldrich were used. For differential scanning calorimetry (DSC) measurements, DMPC was hydrated to achieve a concentration of 2 mM unilamellar vesicles, as described previously.[3] DSC thermograms were generated by heating and cooling from 5–45 °C with a rate of 10 K h^{-1}. Four temperature scans were performed, where the first scan was discarded to obtain a common thermal history of all samples. The measurements were performed on a MicroCal VP-DSC Microcalorimeter (Täby, Sweden). The macroscopically oriented multibilayers of DMPC were prepared according to a previously described procedure.[3,4] The PEO concentration in the water phase of the oriented bilayers was ~2 wt%. A Chemagnetic InfinityPlus NMR spectrometer (Agilent) operating at proton frequencies of 360 MHz was used. The ^{31}P NMR spectra were recorded at 145.703 MHz using single pulse excitation. The ^{1}H NMR diffusion measurements were performed at 359.92 MHz using the stimulated echo pulsed field gradient procedure. A square sample tube containing macroscopically oriented lipid multibilayers was placed in a specifically designed goniometer probe, which enabled the bilayers to be oriented with the bilayer normal at the magic angle (54.7°) with respect to the constant magnetic field of the spectrometer. This causes the dipolar interactions to vanish, resulting in a significant reduction of the line width. The NMR PFG method for measuring lipid lateral diffusion on macroscopically oriented bilayers was described elsewhere.[5] For all measurements, we applied the stimulated echo pulse sequence.[6] The echo amplitude diffusion decay (DD) in the case of a one-component, non-associated liquid is the dependence of the echo amplitude (A) on the pulse sequence parameters (δ, g and t_d) and the self-diffusion coefficient of molecules (D):

$$I = I_0 \exp(-2\tau_2/T_2) \exp(-\tau_1/T_1) \exp[-\gamma^2 g^2 \delta^2 D(\Delta - \delta/3)], \quad (1)$$

where I_0 is the factor proportional to the proton content of the system; T_1 and T_2 are spin–lattice and spin–spin relaxation times, respectively; τ_2 and τ_1 are time intervals in the pulse sequence; γ is the gyromagnetic ratio for protons; g and δ are the amplitude and duration of the gradient pulse, respectively; $t_d = (\Delta - \delta/3)$ is the diffusion time; $\Delta = (\tau_2 + \tau_1)$ is the time interval between the two gradient pulses. In our experiments, g = 1.15 T m^{-1} was set constant and δ was varied in the range of 1.1–8.2 ms, τ_2 = 11 ms and τ_1 = 100 ms. We Fourier transformed the experimental echoes into corresponding sets of spectra and calculated the diffusion coefficient from a non-linear fit of the obtained signal decay. For bilayers oriented at the magic angle, the lateral diffusion coeffi-

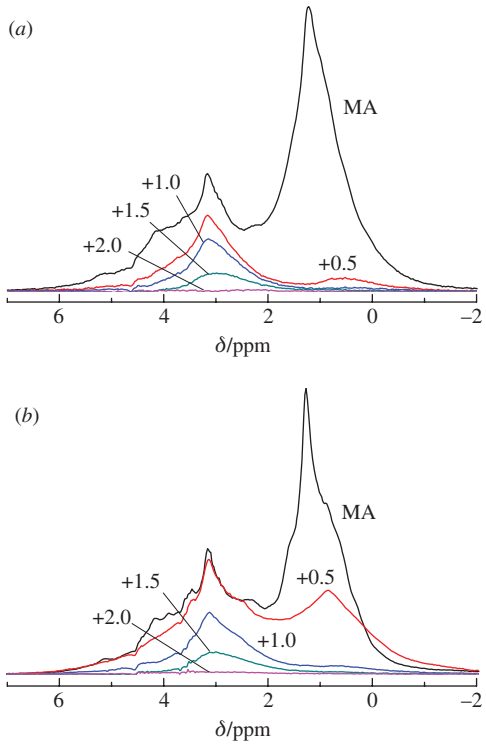

Figure 1 ^{1}H NMR spectra obtained after Fourier transformation of the descending half of stimulated echo of DMPC bilayers (*a*) without and (*b*) with PEO, oriented at the magic angle (black) and at angles subsequently deviating from magic angle by 0.5°. PEO/DMPC weight ratio, 0.02. Temperature, 30 °C.

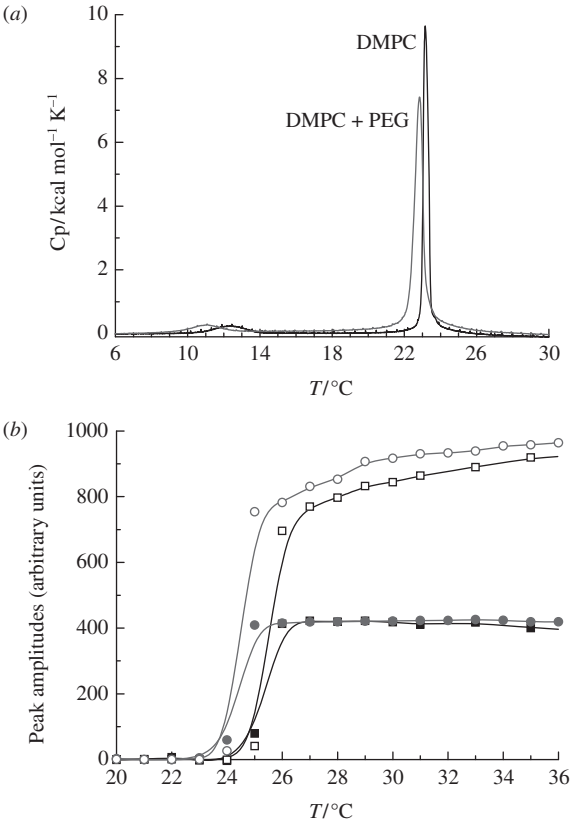

Figure 2 (*a*) DSC thermograms of vesicular 2 mM solutions of DMPC without (black) and with (grey) PEO. PEO/DMPC weight ratio, 0.076. (*b*) Temperature dependences of amplitudes of ^{1}H NMR spectra lines corresponding to choline headgroups (3.1 ppm, solid symbols) and acyl chains (1.3 ppm, open symbols) of DMPC bilayers without (squares) and with PEO (circles) oriented at the magic angle. PEO/DMPC weight ratio, 0.02.

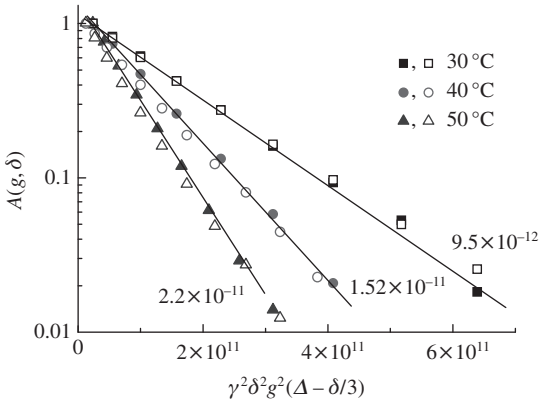

Figure 3 Normalized DDs in the oriented multibilayers of DMPC without (solid symbols) and with (open symbols) PEO. Straight lines indicate the average slopes of the decays with corresponding lateral diffusion coefficients of DMPC in m^2 s^{-1}. PEO/DMPC weight ratio, 0.02.

cient of the lipids (D_L) and the measured self-diffusion coefficient of the lipids are related as $D_L = 1.5D$.[5]

The ^{31}P NMR spectra (not shown) demonstrated that DMPC maintains the lamellar phase in suspended and oriented samples with and without PEO. Figure 1 shows changes in the ^{1}H NMR spectra as a function of the orientation of bilayers near the magic angle. The signal from the lipid chains (1.3 ppm) in DMPC bilayers with PEO [Figure 1(*a*)] decays more slowly than that from the lipid chains in pure DMPC [Figure 1(*b*)]. This validates the worsening orientation of DMPC molecules in the presence of PEO. This loss of orientation agrees with the change in solid-to-liquid phase transitions in the vesicles [Figure 2(*a*)] and the oriented multibilayers [Figure 2(*b*)] in the absence and presence of PEO. For both types of systems, the presence of PEO led to a decrease in the solid-to-liquid temperature of approximately 1 °C with some decrease of the transition cooperativity (broadening of the main DSC peak) and also an increase in the ^{1}H NMR signal amplitude corresponding to the lipid chains, as a result of disordering [because of the increase in T_2 of lipid chains protons, as follows from equation (1)].

Typically, the disordering of lipid chains leads to a rise in the lipid bilayer free volume, which results in an increased lateral lipid mobility.[7–9] A comparison of the DDs for bilayers with (open symbols) and without PEO (solid symbols) in the temperature range of 30–50 °C demonstrates no difference in either the mean diffusion coefficients or the entire DD curves (Figure 3). Thus, the presence of PEO does not change the average free volume of the DMPC multibilayer.

The apparent contradiction between the occurrence of some lipid chain disordering and the lack of any change in the average lipid free volume (and lateral diffusion) in the presence of PEO can be explained by keeping in mind the particular place of the interaction between PEO and DMPC molecules in the DMPC bilayers. PEO, as a hydrophilic molecule, cannot insert deeply into the lipid membrane; therefore, it can only change the local mobility of lipid chains near the transitional hydrophilic–hydrophobic region of the bilayer, somewhat decreasing the strength of the interaction between the lipid chains, which disorders some parts of the lipid chains and slightly decreases the solid–liquid phase transition. At the same time, the ordering of lipid chains closer to the center of the membrane remains undisturbed, preserving the average volume of the lipid bilayer so it remains unchanged. This model of the PEO–biomembrane interaction agrees with the earlier model proposed by Lee *et al.*,[10] based on ESR spectroscopic data.

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References

1 P. Vermette and L. Meagher, *Coll. Surf. B: Biointerfaces*, 2003, **28**, 153.
2 B. R. Lentz, *Eur. Biophys. J.*, 2007, **36**, 315.
3 A. Filippov, B. Munavirov, T. Sparrman, V. Ishmuhametova, M. Rudakova, P. Shriram and S. Tavelin, *Langmuir*, 2011, **27**, 3754.
4 A. Filippov, G. Orädd and G. Lindblom, *Chem. Phys. Lipids*, 2009, **159**, 81.
5 G. Orädd and G. Lindblom, *Magn. Reson. Chem.*, 2004, **42**, 123.
6 E. D. Stejskal and J. E. Tanner, *J. Chem. Phys*., 1965, **42**, 288.
7 W. L. C. Vaz, R. M. Clegg and D. Hallmann, *Biochemistry*, 1985, **24**, 781.
8 A. Filippov, G. Orädd and G. Lindblom, *Biophys J.*, 2007, **93**, 3182.
9 A. Filippov, B. Munavirov, G. Gröbner and M. Rudakova, *Magn. Reson. Imaging*, 2012, **20**, 413.
10 D. K. Lee, J. S. Kim, Y. M. Lee and Y. S. Kang, *Langmuir*, 1998, **14**, 5184.

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