

Bio-Membrane Flexibility Studied in the Presence of Cholesterol and Salt

Living cell membranes are made of phospholipids assembled in two-dimensional bilayers in such a way that the hydrophobic chains are being shielded from the surrounding water as illustrated in Fig. 1. Due to their extreme softness the phospholipid bilayers exhibit thermal undulations in an aqueous environment that give rise to repulsive forces between the cells or between cells and substrates. These forces govern important phenomena in the life process such as cell coagulation and the ability of the cells to sense and react to their surroundings. Here, we report results on the thermal undulations of phospholipid bilayers obtained through the Neutron Spin Echo (NSE) method, the only technique covering the time scale of these motions, 1 ns to 10 ns [1, 2].

The thermal undulations of phospholipid bilayers are strongly dependent on the mechanical properties of the bilayer such as the bending elasticity. Following earlier studies by other methods [3, 4] we have studied the dynamics of a model system, multilamellar vesicles (MLV, see Fig. 1) in water, as a function of temperature, the presence of cholesterol, and the presence of salt. Temperature is expected to influence several properties. Increase in temperature decreases the solvent viscosity and increases the thermal undulation amplitudes. The hydrophobic core of the phospholipid membranes exhibits a sharp phase transition from the crystalline to the liquid phase at a certain temperature T_c that depends on the length of the hydrophobic chains. Above T_c the interior of the phospholipid membrane is much more disordered and is expected

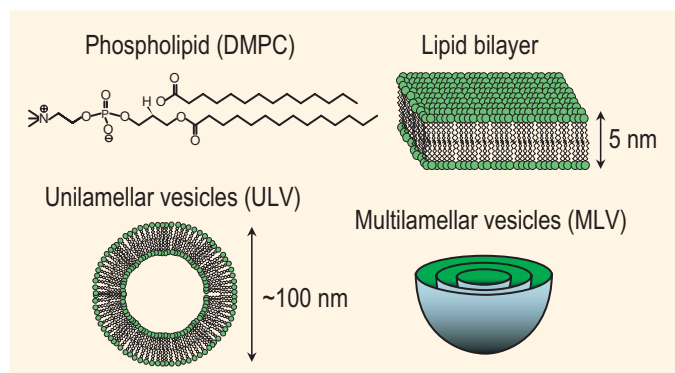


Fig. 1. Illustrations of a typical phospholipid (DMPC), a phospholipid bilayer, a unilamellar vesicle and a multilamellar vesicle.

to be less rigid. Cholesterol, an important constituent of the living cell, nestles in between the fatty tails, changing the mechanical properties of the bilayers and hence their motions. Finally, the presence of sodium chloride as an electrolyte has a screening effect on electrostatic interactions and may affect the thermal undulations of electrostatically charged bilayers.

Three MLV samples are prepared for this study. Sample I (“vesicles”), which serves as a reference, is a MLV made mainly of 1,2-Dimyristoyl-sn-Glycero-3-Phosphocholine (DMPC) in deuterated water (D_2O) to provide scattering contrast. The DMPC vesicles were stabilized through enhanced electrostatic repulsion between the bilayers by addition of a small amount (0.05 mol to 1 mol DMPC) of 1,2-Dimyristoyl-sn-Glycero-3-[Phosphoric-(1-glycerol)]-sodium salt (DMPG), a phospholipid that is structurally similar to DMPC. In sample II (“vesicles & chol”) cholesterol was added in the ratio of 0.3 mol cholesterol to 1 mol of lipids. In sample III (“vesicles & salt”) 0.05 mol L^{-1} of NaCl was added. MLV were prepared by extrusion through a filter with a 200 nm pore size that predetermines their average diameter, as confirmed by dynamic light scattering.

The NSE technique provides a measurement of the real component of the intermediate scattering function in the time domain $I(Q,t)$. Thermal diffusive motions lead to an exponential time decay of $I(Q,t)$. Therefore we have fitted our data for each sample at each temperature with $I(Q,t)/I(Q,0) = \exp(-D_{\text{eff}}Q^2t)$, where the effective diffusion coefficient D_{eff} is a sum of two parts: D_{def} , representing the diffusive motions associated with the fluctuations within each vesicle, the thermal undulations; and D_{tr} , the translational diffusion coefficient of the vesicles in the solution [5]. The latter can be neglected because the vesicles prepared here are quite large, $R \approx 100$ nm, and according to the Stokes-Einstein hydrodynamic equation the corresponding translational diffusion D_{tr} is less than $2.6 \times 10^{-12} \text{ m}^2 \text{ s}^{-1}$. Samples “vesicles” and “vesicles & chol.” were studied at temperatures of 20 °C, 35 °C and 45 °C to cover regions below and above T_c of DMPC which is 24 °C. Sample “vesicles & salt” was measured at 35 °C to study the effect of added electrolyte. Selected results are shown on Fig. 2 and summarized in Table 1.

Table 1. Effective diffusion coefficient of DMPC vesicles at different compositions and temperatures.

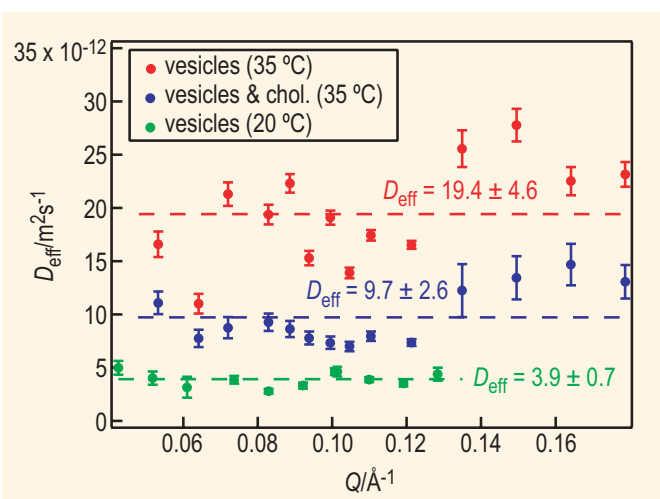
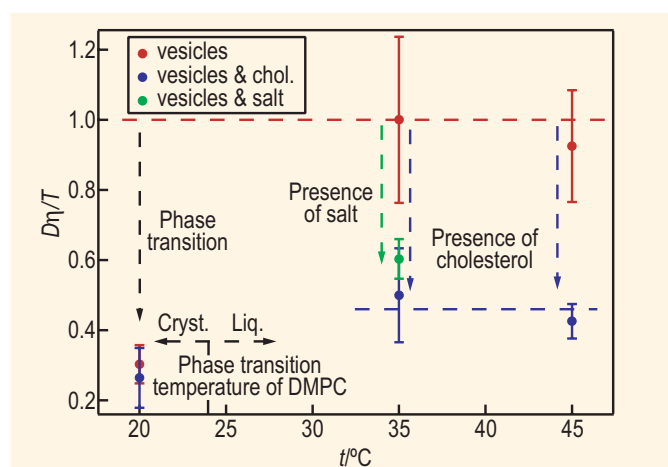
Sample	$t/^\circ\text{C}$	$D_{\text{eff}} \times 10^{12} / \text{m}^2 \text{s}^{-1}$	$\eta_{\text{D}_2\text{O}} \times 10^3 / \text{N s m}^{-2}$	$D_{\text{eff}} \eta / T$
vesicles	20	3.9 ± 0.7	1.25	0.30
vesicles	35	19.4 ± 4.6	0.871	1.00
vesicles	45	22.6 ± 3.9	0.714	0.92
vesicles & chol.	20	3.4 ± 1.1	1.25	0.26
vesicles & chol.	35	9.7 ± 2.6	0.871	0.50
vesicles & chol.	45	10.4 ± 1.2	0.714	0.43
vesicles & salt	35	11.7 ± 1.1	0.871	0.60

To take into account the effect of the solvent viscosity and temperature we have calculated the ratio $D_{\text{eff}}\eta/T$ and normalized it to that at 35 °C. All deviations of the normalized parameter $D_{\text{eff}}\eta/T$ from 1 can be attributed solely to the mechanical properties of the phospholipid bilayer and are not related to changes in the viscosity of the surrounding water.

The analysis of the normalized parameter $D_{\text{eff}}\eta/T$ as a function of temperature leads to the following conclusions (see Fig. 3):

- Scaling the diffusion coefficient D_{eff} that measures the thermal undulations of DMPC bilayers like $D_{\text{eff}}\eta/T$ mitigates the effects of temperature and the viscosity of the surrounding fluid. Above the bilayer interior crystal/liquid phase transition the scaled effective diffusion coefficient for DMPC vesicles remains constant with temperature.
- The bilayer liquid to crystalline phase transition increases the rigidity of DMPC bilayers by a factor of 3 for vesicles and by a factor of 1.5 for vesicles in presence of cholesterol.
- Cholesterol increases the rigidity of the DMPC bilayers in a bilayer-liquid state by a factor of 2 but has no effect on the DMPC bilayers in a bilayer-crystalline state.
- Addition of 0.05 mol L⁻¹ of NaCl reduces the fluctuations of the DMPC bilayers by a factor of 2. This effect can be attributed either to the reduced electrostatic repulsion between the DMPC bilayers or to the effect of the osmotic pressure that can deflate the MLV.

These results are in good agreement with those obtained from other methods [3, 4]. They are a direct confirmation that cholesterol stiffens cell membranes thus reducing the cell — cell repulsions that originate from undulation forces. The significant difference in the thermal undulations below and above the bilayer liquid to crystalline phase transition temperature shows the importance of temperature for the proper functioning of the cell mem-


Fig. 2. Effective diffusion coefficient of DMPC vesicles as a function of the scattering vector Q . D_{eff} reflects mainly the thermal undulations of the phospholipid bilayer.

Fig. 3. Effective diffusion coefficient of DMPC vesicles, scaled by the solvent viscosity and temperature, and normalized to that at 35 °C.

branes. Finally, it has been shown that the NSE method provides a direct means of observing thermal undulation of biological membranes.

References

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