Article

Tuning Membrane Thickness Fluctuations in Model Lipid Bilayers

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ABSTRACT Membrane thickness fluctuations have been associated with a variety of critical membrane phenomena, such as cellular exchange, pore formation, and protein binding, which are intimately related to cell functionality and effective pharmaceuticals. Therefore, understanding how these fluctuations are controlled can remarkably impact medical applications involving selective macromolecule binding and efficient cellular drug intake. Interestingly, previous reports on single-component bilayers show almost identical thickness fluctuation patterns for all investigated lipid tail-lengths, with similar temperature-independent membrane thickness fluctuation amplitude in the fluid phase and a rapid suppression of fluctuations upon transition to the gel phase. Presumably, in vivo functions require a tunability of these parameters, suggesting that more complex model systems are necessary. In this study, we explore lipid tail-length mismatch as a regulator for membrane fluctuations. Unilamellar vesicles of an equimolar mixture of dimyristoylphosphatidylcholine and distearoylphosphatidylcholine molecules, with different tail-lengths and melting transition temperatures, are used as a model system for this next level of complexity. Indeed, this binary system exhibits a significant response of membrane dynamics to thermal variations. The system also suggests a decoupling of the amplitude and the relaxation time of the membrane thickness fluctuations, implying a potential for independent control of these two key parameters.

INTRODUCTION

Lipids are the major component of biological membranes through which a delicate balance of nutrients within and outside the cell is maintained. For a long time, this complex functionality in cell membranes was mainly attributed to membrane proteins while lipid bilayers were overlooked as structureless support matrices. Over the last few decades, however, more focus has been directed to the role of lipids in mediating membrane functions (1–3). Conclusions of recent experimental and numerical studies support a growing consensus that lipid bilayers are far from inert and can have an essential role in determining the function of membrane proteins (4,5). The results of such studies clearly show that the behavior of membrane proteins can be severely compromised depending on their structural response to the thickness and curvature of the host bilayer (1,6,7). Unfortunately, much less is known about the dynamical interactions between proteins and lipid membranes despite the recognition that protein functions are ultimately governed by their local dynamics and that protein binding mechanisms are most likely driven by dynamical synergy between the proteins and the bilayer. Based on the energy landscape of membrane proteins, one would expect the synergistic dynamics to not only be important at the longer timescales of microseconds to milliseconds, but also at the much faster picosecond-to-nanosecond timescales. While these are more difficult to experimentally probe, it is becoming increasingly clear that they play a significant role in protein functionality (8), protein-protein binding (9), and enzyme catalysis (10). Indeed, there is growing realization that a number of biological functions, while being directly linked to slow protein dynamics, have their origins in the faster dynamics through solvent interactions (11) or through a dynamical hierarchy in which faster motions control the slower ones (8). However, the full extent of the role of fast dynamics is still poorly understood due to the experimental difficulties in probing this time regime. Interestingly, these timescales coincide with those of collective thermal fluctuations in lipid bilayers (12,13), which starts to suggest that tuning these fluctuations is critical for medical applications requiring selective macromolecule binding. Of particular interest in this context are membrane thickness fluctuations whose dynamics are on the same timescale as conformational transitions in proteins (8) and that have been associated with other membrane functions such as pore formation (14) and passive permeation (15).

Efforts in studying bilayer thickness fluctuations have been hindered by limitations of experimental techniques

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that can simultaneously access the proper length and timescales. In most cases, the experimental evidence for local membrane fluctuations has been rather indirect (16,17) or inferred from simulations (18,19). More recently, neutron spin-echo (NSE) spectroscopy was effectively used for direct observation of membrane fluctuations in oil-swollen surfactant bilayers (20) and unilamellar lipid vesicles (12). The NSE studies on single-component lipid bilayers with different lipid tail lengths, i.e., DMPC (dimyristoylphosphatidylcholine), DPPC (dipalmitoylphosphatidylcholine), and DSPC (distearoylphosphatidylcholine), revealed some interesting membrane dynamics. Membrane thickness fluctuations were only observable in the fluid phase of the bilayer, implying that such fluctuations are either completely suppressed in the gel phase of the membrane (19) or are slowed down beyond the temporal resolution of the NSE technique. The study also showed almost identical thickness fluctuation patterns (12) in the bilayers, regardless of the length of the lipid molecules used. This invariability in the dynamical behavior relative to lipid tail-length can be attributed to geometrical constraints caused by the minimalistic bilayer composition in this overly simplistic system, which favors uniform bilayer thickness and lateral membrane homogeneity.

However, if the lateral and/or normal symmetry in the membrane is broken, as in biological membranes, one would expect the change in the membrane energetics to drive the system into a more dynamic state. In this study, we investigate the effect of lipid tail-length mismatch in breaking the symmetry and regulating membrane dynamics. We consider as a model system a mixture of length-mismatched DMPC and DSPC molecules characterized by four additional carbon atoms in DSPC tails. The two molecules experience distinct transition temperatures, T_m, at which the tails change from a stiff stretched configuration (gel phase) to a flexible, more coiled configuration (fluid phase). For tail-deuterated (dt) lipids, the transition temperatures are T_m (dtDSPC) = 50.5°C and T_m (dtDMPC) = 20.5°C (12), yielding a broad thermal range of gel-fluid coexistence in DMPC/DSPC mixtures (21,22). Additionally, the two lipid molecules experience dramatic changes in their tail-length mismatch of $\approx$1 nm in the fluid and gel phases and $\approx$2 nm in the gel-fluid coexistence phase (12). These remarkable disparities in the transition temperatures and the tail-length mismatch render DMPC/DSPC mixtures an ideal model for these investigations. The broadest gel-fluid coexistence phase (21) is obtained for an equimolar mixture of DMPC and DSPC, which is the focus of this study. Due to neutron scattering sensitivity to deuterium labeling, we employ selective deuteration to probe different bilayer features. For example, thickness fluctuation parameters are best obtained from tail-contrast-matched vesicles of primarily perdeuterated-tail forms of DMPC and DSPC such that the tail region in the bilayer contrast-matches the carrier solvent (D_2O) (see Table S1 in the Supporting Material). This is the main system used in this work unless otherwise noted.

MATERIALS AND METHODS

Materials

The lipids were purchased from Avanti Polar Lipids (Alabaster, AL) and used without any further purification. The equimolar mixture of DMPC and DSPC was prepared as discussed in Section S1 in the Supporting Material. The lipids were weighed in powder form and dissolved into chloroform for good dissociation, after which the chloroform was evaporated under flow of nitrogen gas. The lipid mixture was then put under vacuum overnight to ensure full evaporation of the chloroform. The dried lipids were then dispersed in D_2O above $T = 55$°C. Unilamellar vesicles were obtained by extruding the mixture, at $T = 60$°C, through polycarbonate filters with pore sizes 400, 200, and 100 nm, consecutively. The extruded solutions were kept above $T = 55$°C until measured.

Methods

Density measurement

To determine the transition temperatures of the mixed lipid bilayers, the solution density was measured using a model No. DMA5000 density meter (Anton Paar, Ashland, VA). Knowing that the lipid molecular density changes dramatically at the gel-fluid transition temperature, temperature-dependent density measurements were conducted over a temperature range from $T = 65$°C to $T = 15$°C with a step of 0.2°C.

Small-angle neutron scattering measurement

Small-angle neutron scattering (SANS) experiments were performed at the NG7-30-meter SANS at the National Institute of Standards and Technology (NIST) (23,24). The selected q range was from 0.03 to 5 nm^{-1} with the use of a 0.6-nm neutron wavelength. The samples used were put in 1-mm-thick quartz cells. The sample temperature was decreased from $T = 65$°C to $T = 15$°C using a circulation bath with an accuracy better than 0.5°C. The raw data were processed via an established reduction protocol to obtain absolute scattering intensity in units of cm^{-1} (25). Data fitting was performed using the software SasView (www.sasview.org).

Small-angle x-ray scattering measurement

Small-angle x-ray scattering (SAXS) measurements were performed at the x27c beam line at Brookhaven National Laboratory (Upton, NY). The accessed q range was 1 to 3.5 nm^{-1} using a wavelength of 0.137 nm at a sample-to-detector distance of 95.7 cm. The scattered photons were collected by a charge-coupled device camera. The detector distance and q value were calibrated using silver behenate. The samples were measured in a 1-mm-diameter capillary tube taped to a temperature-controlled stage. The measurements were done over a temperature range of 65–25°C with an accuracy of $\pm$1°C. Data reduction, including background subtraction, was performed using the xPOLAR software developed at Precision Works NY, Inc. (East Setauket, NY).

NSE measurement

NSE experiments were performed using the NG5-NSE spectrometer at NIST (26) at neutron wavelengths of 0.6 and 0.8 nm with a wavelength spread of ~18%. The measured q ranges spanned from 0.4 to 1.8 nm^{-1} and Fourier times, t, from 0.05 to 40 ns. The tail-contrast-matched sample was measured in a 4-mm-thick cell and the hydrogenated sample in a 1-mm-thick cell. The temperature was varied from $T = 65$°C to $T = 15$°C using a circulating bath with an accuracy better than $\pm$0.1°C. The obtained NSE signals were reduced to the intermediate scattering function using the software DAVE (27), which properly accounts for background and resolution corrections.

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