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Dynamics of topological defects in the $L_{\beta'}$ phase of 1,2-dipalmitoylphosphatidycholine bilayers

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Abstract

We use the optical birefringence of 1,2-dipalmitoylphosphatidycholine bilayers (DPPC) in the gel ($L_{\beta'}$) phase to study recombination dynamics of topological defects. The birefringence of anisotropic thin films, such as the $L_{\beta'}$ phase of DPPC bilayers, is related to their molecular polarizability, different on the heads and the acyl chains. When the sample is cooled down into the $L_{\beta'}$ phase, a period of rapid recombination (taking place over a few seconds) is followed by slow dynamics with metastable states existing in excess of several minutes. After this, either another metastable state or a truly stable state remains where no further change is observed, although a spatially non-uniform distribution of the orientation of the birefringence remains. We compare our results with a model for the free energy and the dynamics of the lipid bilayer in the gel state, finding good qualitative agreement.

Keywords: Lipid bilayers; Topological defects; Dynamics; Near-field microscopy

1. Introduction

Supported lipid bilayers have attracted much interest as an idealized model system in biophysics research. They serve as simple models for gaining insight into real biomembranes and the basic structure-function role they play in intracellular signaling [1] and also hold the promise of important medical applications, such as drug delivery [2]. A subject of considerable interest is the thermodynamic properties of lipid bilayers, both from the fundamental and applied points of view. It does not come as a surprise then that over the last twenty five years, several methods for studying these properties have been developed and refined. On the one hand, methods that provide microscopic or molecular information include neutron diffraction [3], X-ray diffraction [4], and electron-spin resonance [5], but in general they suffer from two drawbacks: The information is not local and extensive modeling is needed to relate the data to macroscopic properties. On the other hand, information on the macroscopic level is obtained by means of differential scanning calorimetry [4], analysis of shape fluctuations [6–8], atomic force microscopy [9], and optical dynamometry [10]. These approaches provide a direct measurement of the bilayer response, but they are often intrusive, altering its state.

Most pure optical methods, while minimally intrusive, are limited by resolution. Ensemble methods, such as fluorescence recovery after photobleaching [11], cannot resolve the local, heterogeneous diffusive behavior existing in these systems. While confocal [12] and phase [13] microscopies provide enhanced spatial resolution (~300 nm), still far from molecular level detection. The need to have a fast, sensitive scheme that probes locally the lipid bilayers fueled the development of single molecule diffusion techniques. One such technique relies on chemically binding a particle to a specific target [14]. The disparity in the relative sizes between the particle (~100 nm) and the molecule (~1 nm) is too large to yield the unperturbed dynamics of the molecule. Another approach, optical tagging with far field observation, proved to be tremendously useful and versatile, allowing for the identification of the diffusion characteristics of individual

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fluorescent molecules [15,16]. The use of this technique, however, has to be made compatible with a low density of tagged molecules to assure that only one molecule is being excited within the spatial resolution of the microscope (typically 0.3 μm). Furthermore, the background noise is relatively large, since the whole sample is unselectively illuminated, and molecules are observed every ~10 ms.

In our group we have used a near-field scanning optical microscope (NSOM) to study DPPC [17,18]. The NSOM has the advantage that allows the implementation of most far-field optical approaches while not suffering from the resolution limits encountered in conventional far-field approaches. With aperture based NSOM, a small aperture is scanned in close proximity to the sample. The sample is illuminated through the aperture (with typical dimensions ~50 nm, much smaller than the wavelength of light) and the resolution is mainly determined by the size of the aperture [19]. In this paper we use a near-field scanning optical microscope (NSOM) [17–19], to investigate DPPC lipid bilayers in the gel phase. The effect on the optical properties of the inherent anisotropy of lipid molecules is used to map the lateral organization of the membrane. The many degrees of freedom of the acyl chains in the lipid molecules and their interactions are responsible for a rich phase diagram. Single component lipid bilayers form a solid-like gel state at low temperature that, upon warming, turns into a liquid crystalline one. Although the details of the phase transition are lipid specific, it has been found that a variety of bilayers adopt several structurally different arrangements dependent upon temperature and hydration [20]. Using DPPC bilayers, we show results with a spatial resolution of ~50 nm and, furthermore, we experimentally show the dynamics of topological defects formed when cooling the sample from the L_α liquid crystalline to the $L_{\beta'}$ gel state.

2. Experimental details

2.1. Sample preparation and characterization

A similar approach to the one used in Ref. [17] was used. Supported bilayers of DPPC were prepared by fusion of sonicated unilamellar vesicles (SUVs) onto a glass surface [21]. While also mica was used to study the formation of the lipid bilayer, the intrinsic birefringence found in mica precludes using it as a substrate for these studies. SUVs (1.5–2 mg/ml lipid) were made in 70 mM NaCl–20 mM NaH_2PO_4 (pH 7). They were passed 20 times through either a 100, 50, and 30 nm filters or a drop (between 30 and 60 μl) was deposited on a clean microscope slide. The sample was then incubated overnight at 4 °C, followed by 1.5–2 hr at 60 °C. Excess lipids and buffer were then washed off using deionized (DI) water. For atomic force microscopy (AFM) the samples were transferred to a fluid cell with DI water, while NSOM data were acquired in a chamber at 100% relative humidity. All AFM measurements were performed at 20 °C a few hours after sample preparation. One such measurement is shown in Fig. 1, which shows regions in the sample were the glass is exposed, and regions of continuous DPPC. The difference in height between the exposed glass substrate and the top of the DPPC bilayer is used to measure the height of the lipid bilayer. The average over many samples yielded $t = (5.3 \pm 0.1)$ nm, in good agreement with the thickness of a single DPPC bilayer in the gel state [20]. No appreciable curvature of the membranes was observed.

For the NSOM images, the samples were heated up to ~55 °C, into the L_α phase. They were kept at this temperature for about 30 min and then rapidly cooled to the desired temperature. While the final temperature was varied between (20 ± 2) °C and (35 ± 2) °C in 5 °C intervals, no significant differences were observed. All the results reported here correspond to a final temperature of (25 ± 2) °C. It was checked that none of the features observed in the $L_{\beta'}$ phase were observed in the L_α phase. This was accomplished by doing the experiment at high temperatures or by adding cholesterol to the DPPC and working at room temperature, as in Ref. [17].

2.2. Experimental setup and methods

The optical contrast in the $L_{\beta'}$ phase [22,23] arises from the anisotropic index of refraction of phospholipid bilayers [23]. As described in Ref. [17], the analysis of the change in polarization of the light as it crosses the lipid bilayer can be used to extract the retardance $\Delta\varphi$ of the bilayer, where

$$\Delta\varphi = \frac{2\pi}{\lambda} t(n_e - n_o), \tag{1}$$

$\lambda = 632.8$ nm is the wavelength of the He–Ne laser used, n_e is the index of refraction of the extraordinary ray, and n_o is the index of refraction of the ordinary ray. From $\Delta\varphi$ the birefringence S can be obtained [17]. The polar orientation ϕ of the acyl chains (i.e. the projection of the direction of the acyl chains onto the plane of the membrane) is defined with respect to the direction of the first polarizer on the optical setup. We repeated these measurements in this paper, but also used an approach that directly yields the birefringence and the polar orientation [24]. A schematic of the experimental setup is shown in Fig. 2. Light from the He–Ne laser is passed through a linear polarizer (LP, with transmission axis, TA, defined to be at 0°), a photoelastic modulator (PEM, which axis of varying index of refraction is at 45°), a quarter wave plate (QWP with slow axis, SA, at 0°), coupled into a single mode optical fiber which in turn goes through a universal polarizer and the NSOM probe. Light from the NSOM probe, after going through the sample, passes through another QWP (SA 0°), and LP (TA − 45°), and then is collected by a PIN Si diode. The universal polarizer is adjusted such that, in absence of the PEM, light from the NSOM probe is circularly polarized. The presence of the PEM, modulated at its first resonance frequency ω_o, yields a signal at the detector at DC, and at all the harmonics of ω_o. It can be shown that the intensity at the detector is [24]