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Infrared insights into the effect of cholesterol on lipid membranes

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ABSTRACT

Utilizing a combination of time-resolved Infrared (IR) spectroscopies, we are able to probe the effects of a small biological molecule (cholesterol) on the dynamics of lipid membranes from picoseconds to milliseconds. By monitoring the ultrafast dynamics of the system with multi-dimensional IR spectroscopy, we are able to resolve the influence cholesterol has on the electric field fluctuations inside the membrane. We use temperature-jump (T-jump) spectroscopy to extend our experimentally-observable time window beyond the vibrational lifetime of our probe and find that the presence of cholesterol introduces significant inhomogeneity into the relaxation rates of the system. This approach provides new insight into the interaction between cholesterol and lipids, while also demonstrating the utility of this combined IR spectroscopic toolbox for studying multiscale dynamics.

1. Introduction

Cholesterol is one of the primary components of biological membranes [1]. It influences many of the fundamental structural and dynamic properties of membranes and plays an important functional role for membrane proteins [2]. The mechanical properties (bending modulus, compression modulus [3]), structural properties (lipid order and packing [4]) and dynamic properties (diffusion rates [5], phase transition behavior [6]) are all modulated by the presence of cholesterol. Many membrane proteins require not just the presence or absence of cholesterol to function, but rather are observed to have an optimal cholesterol level [2]. Though the functional effect of cholesterol may be readily demonstrated, understanding the molecular origins of this effect is a more challenging proposition. Key to understanding the effect of cholesterol on membranes and membrane proteins is understanding how the multiple modes of action of cholesterol, separated by many decades in length- and timescales, relate to one another.

Time-resolved infrared (IR) spectroscopy is a powerful tool for generating structural "snapshots" of systems with sub-picosecond time-resolution and functional group-specificity. IR spectroscopy has been successfully used to probe many aspects of membrane structure and dynamics, from the dipole potential of membranes [7,8] to the phase transition behavior of membranes [9,10]. Two-dimensional IR spectroscopy (2D IR) is a technique which uses sub-picosecond IR pulses to probe the correlations between vibrations in a time-resolved fashion, and has been successfully applied to study the influence of cholesterol on the fast dynamics of the hydrophobic region of the membranes [11–13].

The experimentally observable window in 2D IR experiments, however, is limited by the lifetime of the target vibration; in the case of carbonyl stretches, this lifetime is <1.5 ps. To extend the range of observable dynamics, we introduce a second set of experiments – temperature jump (T-jump) spectroscopy. Here, the sample is rapidly heated by a high-energy nanosecond pulse and the response of the system is probed by the 2D IR pulse sequence [14]. In this experiment, the range of observable dynamics is limited by the duration of the elevated temperature (milliseconds), rather than the vibrational lifetime of the carbonyl group.

This suite of experiments, spanning the picosecond to millisecond range, is used to study the effect of cholesterol on the dynamics of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) membranes with the aim of developing an understanding of how cholesterol interacts with the surrounding lipids on different time- and length-scales. In a recent set of experiments, we described a series of dynamical properties of DMPC lipid bilayers as observed through the vibrational spectroscopy of its ester groups. Picosecond fluctuations of hydration waters and membrane electrostatic fields were observed with 2D IR spectroscopy [15], and the nanosecond-to-microsecond melting dynamics of these membranes were observed following a T-jump induced gel-to-fluid phase transition [16]. This manuscript describes how these DMPC

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dynamics are modified as a result of introducing cholesterol into the lipid bilayer.

2. Materials and methods

The sample preparation protocols, the experimental methods used for 2D IR spectroscopy and temperature-jump experiments, and the analysis methods used for singular value decomposition melting curves have been described in detail previously [15,16]. In the following we add the new details relevant to studies with cholesterol.

2.1. Sample preparation

Two different lipid samples composed of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC, Anatrace) and 10 mol% cholesterol (Sigma Aldrich) were prepared.

2.1.1. Hydrated bilayers

Hydrated lipid bilayers were prepared by co-dissolving DMPC and cholesterol in chloroform and drying this solution on a CaF_2 window. This dried sample was hydrated with D_2O at a 10:1 water:lipid ratio. This sample was heated to 40 °C (above the lipid phase transition temperature), and the procedure outlined in Ref. [12] was used to prepare aligned bilayer stacks. Aligned bilayer samples were required for the 2D IR spectra in order to minimize scatter from the lipid bilayers. 2D IR waiting time data was collected at 40 °C (ensuring the membrane is in the fluid phase) to further minimize scattered light.

2.1.2. Extruded vesicles

The low D_2O concentration of the hydrated bilayers makes them unsuitable for T-jump experiments because the mechanism for increasing temperature involves absorption of an intense nanosecond pulse by the solvent. To prepare samples suitable for T-jump experiments, the DMPC and cholesterol were co-dissolved in chloroform, before being dried under a stream of N_2. This dried lipid film was then dissolved in D_2O to a concentration of 10 mM (with respect to DMPC). Samples were held at 10 °C above the lipid melting temperature for 1 h before vortexing and sonication to ensure a homogeneous sample. Samples were extruded through a 100 nm membrane to form Large Unilamellar Vesicles (LUVs).

2.2. IR spectrometer

2.2.1. 2D IR spectrometer

The 2D IR spectrometer used in this study has been described in detail elsewhere [15]. 2D IR experiments were performed with 90 fs pulses centered at 1730 cm^{-1} in the forward box geometry using heterodyne detection on a HgCdTe array detector. Fourier transform absorptive spectra of the ester carbonyl lineshape were measured as a function of waiting time (T) between excitation and detection periods.

2.2.2. T-Jump spectrometer

For the T-jump experiments described below, a 10 ns, 20 mJ pulse resonant with the OD stretch-overtone vibration ($\lambda = 1.9$ μm) results in an increase in temperature of 10 °C within <10 ns which persists for several milliseconds. The final temperature of the T-jump is determined by the change in absorption of the broad D_2O bend-libration combination band at 1550 cm^{-1}. The delay between the T-jump pulse and the 2D IR pulse sequence is electronically controlled, allowing sampling of timescales from nanoseconds to many milliseconds [14]. To follow transient T-jump kinetics, we measured T-jump induced changes to the complex Heterodyned Dispersed Vibrational Echo (HDVE) spectrum. To facilitate comparison with the temperature-dependent FTIR spectra, the T-jump data is presented as the difference between the absolute-valued HDVE spectra after and before the T-jump pulse, referred to herein as the t-aHDVE.

2.3. MaxEnt iLT analysis

Analysis of the T-jump data is challenging; the number of timescales present in the system is not known *a priori*. If the rates in the system remain static after the T-jump, then the underlying rate distribution could, in principal, be determined by an inverse Laplace transform (iLT) from the time-domain to the rate-domain. This would eliminate the need to presuppose the number of timescales in the system, and would even capture more exotic effects arising from non-Gaussian distributions of rates (such as stretched-exponential time-domain behavior). However, the iLT is ill-posed in the presence of real, noisy data; noise in the time-domain traces will result in significant alterations to the determined rate distribution, rendering a simple numerical iLT on the T-jump data a fruitless endeavor.

Regularization methods provide some additional constraint to the numerical iLT to stabilize the recovered solution with respect to the presence of noise and mitigate the ill-conditioned nature of the inverse transform. The form of the constraint determines the type of regularization; here, we use a variant known as Maximum Entropy (MaxEnt) regularization [17,18], where the entropy of the recovered distribution is calculated from

$$S = -\sum_i x_i \left(\ln\left(\frac{x_i}{X_i}\right) - 1 \right)$$

where x_i is the i^{th} element of the recovered rate distribution and X_i is the i^{th} element of some assumed prior. Typically (and as is the case here), the prior is taken to be a flat distribution with a small, but finite, initial value, so as to impose the minimum number of additional assumptions on the data.

To implement the MaxEnt iLT, the function F is minimized where

$$F = \eta\chi^2 - S$$

and

$$\chi^2 = \frac{1}{N}\sum_k^N \frac{(I_{expt}(t_k) - I_{calc}(t_k))^2}{\sigma_k^2}.$$

$I_{calc}(t_k)$ is the time trace calculated from the distribution, and σ_k^2 is the noise estimate of the data. Though it may seem at first that there are two free parameters in this scheme, σ_k^2 and η, the parameter η is a Lagrangian multiplier which should be used to constrain the solution $\chi^2 = 1$ (*i.e.* the difference between the experiment and recovered solution is only due to noise). In practice, we take σ_k^2 to be independent of k. In the t-aHDVE experiments, we are able to estimate the noise from the transient data where the 2D IR pulse sequence arrives *before* the T-jump pulse (*i.e.* where the transient signal should be zero).

Note that the form of the entropy requires that the coefficients in the rate distribution be positive; this poses a problem for kinetic traces where the rate coefficients have opposite signs. To address this problem, we adopt the same approach outlined in Ref. [17] where positively-signed and negatively-signed distributions are calculated and then summed together.

The MaxEnt iLT procedure may be repeated for different detection frequencies, allowing us to build up a 2D map of the distribution of rates as a function of vibrational frequency. This has been previously used to extract the spectrum associated with a