Dielectric relaxation change of water upon phase transition of a lipid bilayer probed by terahertz time domain spectroscopy

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We investigate the influence of the 1, 2-ditetradecanoyl-sn-glycero-3-phosphocholine lipid bilayer phases on the water reorientation dynamics with terahertz time domain spectroscopy. The phase of the lipids was controlled by the temperature in the range of 14–35 °C. During the gel-to-fluid phase transition, the hydration water ratio drastically changed from 0.3 to 0.6. The absorption coefficient of the hydration water increased with the temperature in the gel phase and then decreased in the fluid phase. The dielectric relaxation time of the lipid solution decreased initially but then increased after the phase transition. This indicates that the hydration water reorientation dynamics are restricted by lipids and that this phenomenon is pronounced in a biologically relevant fluid phase.

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I. INTRODUCTION

The cell membrane forms a stable barrier between the inside and outside of the cell. However, this membrane is not merely a physical barrier. The cell membrane, through ion channels and pumps, exchanges ions and biological molecules from its environment and maintains the balance of the cell.1,2 The lipid bilayer is a major component of the cell membranes. Research on the hydration of the lipid bilayer has intrigued many researchers, as water-lipid interactions play a key role in the stability of the bilayer,3 affect the permeation of water molecules through the hydrophobic core of bilayers,4 and influence the functioning of the membrane.5 Thus far, the structural and dynamical properties of hydration water molecules have been studied with various techniques, such as nuclear magnetic resonance,6,7 neutron scattering,7–9 and Fourier transform infrared spectroscopy (FTIR).10

Recently, terahertz (THz) spectroscopy has been proposed for observing ultrafast hydration water dynamics.11,12 Water molecules form labile hydrogen-bond collective networks which are constantly changed on a picosecond time scale (1 ps−1 = 1 THz).13,14 The constant breaking and forming of hydrogen-bonds results in fluctuations of the water dipole moments, and the THz spectrum is sensitive to the motion. As a consequence, THz spectroscopy can measure subtle changes of water molecules’ reorientation dynamics by the presence of solutes.13 With terahertz time-domain spectroscopy (THz-TDS), Hishida and Tanaka recently revealed the long-range hydration effect of lipids on the sub-ps (10−12 s) time.15 The time scale measured in previous studies7,8 was relatively slow (10−9 ∼ 10−11 s) such that it only observed more tightly bound short-range water molecules.

In spite of the great effort to understand the lipid hydration, a clear picture about the relationship between hydration water dynamics and lipid dynamics is still lacking. To investigate these relationships we assess the lipid dynamics and hydration water dynamics at different temperatures. We measure the temperature-dependent absorption coefficient of the lipid solution. Finally, we show that the hydration water reorientation dynamics restricted by lipids and this phenomenon is prominent in biologically relevant fluid phases.

II. EXPERIMENTAL METHOD

The 1, 2-ditetradecanoyl-sn-glycero-3-phosphocholine (DMPC) lipids (Fig. 1) were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL) in powder form and were used without further purification. The DMPC powder was dissolved in pure water to form multilamellar vesicles. The lipids were fully hydrated15 (the molar ratio: [H2O]/[DMPC] = 80) to avoid unusual properties from arising in the partially hydrated condition.16 Because the concentration of the sample is well above the critical micelle concentration (CMC; 6 nM),17 the lipid molecules which exist in monomer form are negligible. The lipid solutions were placed in a liquid cell with a 50-um-thick Teflon spacer between two z-cut quartz (CaF2) windows for the THz-TDS (FTIR) measurements (Figs. 2(a) and 2(b)). The temperature of the DMPC solutions was controlled and maintained in a variable temperature cell holder (Specac) (Fig. 2(c)). The lipid film was prepared with a DMPC solution in chloroform which was deposited on the quartz window. After the evaporation of the chloroform, the DMPC film remained.18

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THz-TDS was used to measure the absorption coefficient of the fully hydrated lipids and the lipid films. The experimental setup is described elsewhere. A 3.2 W Ti:sapphire regenerative laser amplifier system (Coherent Inc.) with a center wavelength of 800 nm and a repetition rate of 1 kHz was used as a source of the THz wave. The pulse width is \( \sim 180 \) fs (FWHM). A 10×10 mm (110) ZnTe crystal with a thickness of 1 mm was used for the generation of the fs-THz wave via optical rectification. This THz electric field was mapped out by electro-optic sampling method in another 1 mm thick (110) ZnTe nonlinear crystal. Between the emitter and the detector, off-axis parabolic mirrors were used to collimate the THz wave irradiating the liquid cell.

To observe the temperature-dependent CH\(_2\) symmetric stretching mode change of the lipid, the mid-IR absorbance spectra were recorded from 4500 to 1000 cm\(^{-1}\) with a Varian 7000e FTIR spectrometer.

III. RESULTS AND DISCUSSION

Figure 3 shows the THz field amplitude as a function of time after the transmission of the THz pulses through an empty liquid cell (solid line, \( T_{\text{ref}} \)) and through a cell filled with water (dashed line, \( T_{\text{sam}} \)). The inset displays the corresponding spectra. From these spectra, we obtain the transmission function \( T \), which is composed of the Fresnel coefficients, decay function, and multiple reflections inside the sample

\[
T = \frac{T_{\text{sam}}}{T_{\text{ref}}} = \frac{(\hat{n}_q + n_{\text{air}})^2 \hat{n}_{\text{sam}} \exp \left( i \frac{\omega d}{c} (\hat{n}_{\text{sam}} - n_{\text{air}}) \right)}{n_{\text{air}} (\hat{n}_q + \hat{n}_{\text{sam}})^2} \sum_m \left[ \frac{\hat{n}_q - \hat{n}_{\text{sam}}}{\hat{n}_q + \hat{n}_{\text{air}}} \exp \left( i \frac{\omega d}{c} (\hat{n}_{\text{sam}}) \right) \right]^2 \sum_m \left[ \frac{\hat{n}_q - n_{\text{air}}}{\hat{n}_q + n_{\text{air}}} \exp \left( i \frac{\omega d}{c} (n_{\text{air}}) \right) \right]^2 m.
\]

(1)

Here, \( T_{\text{ref}} \) and \( T_{\text{sam}} \) are the transmission amplitude of the empty liquid cell and of the cell with the sample, \( \hat{n}_q \) and \( \hat{n}_{\text{sam}} \) are the complex refractive index of the quartz window and the sample, \( d \) denotes the sample thickness, \( \omega \) is the angular frequency, \( c \) is the speed of light in air, and \( m \) is number of multiple reflections inside the sample within the given time window. We get \( \hat{n}_q \) from another independent measurement. Then, we obtain the absorption coefficient of the sample

\[
\alpha = \frac{2 \omega \text{Im}(\hat{n}_{\text{sam}})}{c},
\]

(2)

where \( \text{Im}(\hat{n}_{\text{sam}}) \) indicates the imaginary part of the sample's complex refractive index.

It is well known that lipids experience gel-to-fluid phase transitions at their phase transition temperature (\( T_m \)). This temperature is determined by several factors, such as the composition, chain length, and hydration state of the lipids. The phase transition temperature of fully hydrated DMPC is known to be in the range 21–25°C. Below \( T_m \), lipids are in a highly ordered gel phase and the van der Waals interactions between the adjacent hydrocarbon chains reach their maximum. At temperatures above \( T_m \), the chains of lipids are relatively disordered and in constant motion.

FIG. 1. The chemical structure of DMPC studied in this experiment.

FIG. 2. (a) Liquid cell with PTFE spacer sandwiched between z-cut quartz windows. (b) Assembled liquid cell is inserted in a (c) variable temperature cell holder.
To check the phase transition of the DMPC studied here, we obtained the temperature-dependent IR spectrum in the mid-infrared region. FTIR spectroscopy in the mid-infrared region reveals the \( \text{CH}_2 \) symmetric stretching mode of the lipid hydrocarbon chains.\(^{24}\) The \( \text{CH}_2 \) symmetric stretching mode frequency depends on the phase of the lipids. We calculated the difference between the absorbance spectra of the DMPC solution and that of bulk water (Fig. 4). Below 20° C, the stretching mode frequency was 2850 cm\(^{-1}\). However, as the temperature increased, the frequency was shifted to 2852 cm\(^{-1}\). This result is consistent with the well-known fact that gel-to-fluid phase transition is observed by the \( \text{CH}_2 \) symmetric stretching mode frequency shift.\(^{24}\) We conclude that below 20° C the lipids are in the gel phase, and above 22° C they are in the fluid phase.

Water molecules form hydrogen-bond networks. The constant forming and breaking of hydrogen-bonds on the picosecond time scale (1 ps\(^{-1}\)), which is connected to the reorientation dynamics of water molecules, can be sensitively detected by THz-TDS. THz-TDS measurements of hydrated lipids at different temperatures enable us to understand the reorientation dynamics of hydration water molecules, which directly interact with the lipid molecules, depending on the phase of the lipids. Figure 5 presents the absorption coefficient of water (solid squares), the lipid solution (empty squares), and the lipid film (solid circles) at 1 THz with different temperatures. The solid lines are the guides for the eyes. Other frequencies studied (0.3–1.5 THz) showed similar temperature-dependency characteristics. The absorption coefficient of water increases with the temperature. The absorption coefficient of the lipid solution also increases as the temperature increases in the gel phase. In the fluid phase, however, the absorption coefficient decreases with the temperature. This temperature dependent THz absorption of the lipid solution cannot be explained by lipid and bulk water (Fig. 5) indicating a nontrivial contribution from water molecules around lipid molecules. Therefore, we continue our analysis with a three-component model, which assumes a distinct absorption coefficient of the water around lipid.\(^{11}\)

In this model, the absorption coefficient of hydrated lipid is described as a sum of volume-weighted average of the absorption of the lipid, the hydration water, and the bulk water

\[
\alpha(\omega)_{\text{solution}} = \frac{V_{\text{lipid}}}{V}\alpha(\omega)_{\text{lipid}} + \frac{V_{\text{hydration}}}{V}\alpha(\omega)_{\text{hydration}} + \left(1 - \frac{V_{\text{lipid}}}{V} - \frac{V_{\text{hydration}}}{V}\right)\alpha(\omega)_{\text{bulk}}. \tag{3}
\]

Here, \( \alpha(\omega)_{\text{solution}} \), \( \alpha(\omega)_{\text{lipid}} \), \( \alpha(\omega)_{\text{hydration}} \), and \( \alpha(\omega)_{\text{bulk}} \) are the absorption coefficient of the lipid solution, the absorption coefficient of the lipid, the absorption coefficient of the hydration water, and the absorption coefficient of the bulk water. \( V \), \( V_{\text{lipid}} \), and \( V_{\text{hydration}} \) are the total volume probed by the terahertz beam, the volume of the lipid molecules, and the volume of the hydration water molecules. \( \alpha(\omega)_{\text{solution}} \), \( \alpha(\omega)_{\text{lipid}} \), and \( \alpha(\omega)_{\text{bulk}} \) are determined from the THz-TDS experiment. We fixed \( V \) and \( V_{\text{lipid}} \) and adjusted \( \alpha(\omega)_{\text{hydration}} \) and \( V_{\text{hydration}} \) in a nonlinear least-squares fitting. The calculated \( \alpha(\omega)_{\text{hydration}} \) at 1 THz is shown in Fig. 6. In the gel phase, the absorption coefficient of the hydration water increases with the temperature,
like the bulk water. However, in the fluid phase the absorption coefficients scarcely change with the temperature, and becoming even lower than that of the gel phase. This shows that the property of hydration water, which is confined in nanoscale structure, resulted the temperature dependent behavior of the lipid solution.

The number of hydration water molecules depends on the lipid phase. Fully hydrated phosphatidylcholine lipids such as DPPC are known to have \(~4 (~9)\) hydration water molecules per DPPC in the gel (fluid) phase. This means that the interaction between the water molecules and the lipid molecules is stronger in the fluid phase. Figure 6(b) shows that the hydration water ratio drastically changes from 0.3 to 0.6 during the gel-to-fluid phase transition. This strong interaction restricts the motion of the water molecules, as represented by the reduced absorption coefficient of the lipid solution in the fluid phase.

A reorientation of the dipole moment is the dominating mode at THz frequency region studied here (0.3–1.5 THz). We restrict our analysis to frequencies up to 1.5 THz to avoid a hydrogen-bond stretching mode at \(~5.4\) THz, and a (very weak) hydrogen-bond bending mode at \(~1.8\) THz. One of the most commonly used models describing dielectric relaxation is the Debye model. For water, we expect two different types of relaxation:

\[
\varepsilon(\omega) = \varepsilon_\infty + \frac{\varepsilon_s - \varepsilon_0}{1 - i\omega\tau_1} + \frac{\varepsilon_0 - \varepsilon_\infty}{1 - i\omega\tau_2}.
\]

Here, \(\tau_1\) is the main relaxation time, \(\tau_2\) is the fast relaxation time, \(\varepsilon_\infty\) indicates the dielectric constant at the high-frequency limit, and \(\varepsilon_s\) is the dielectric constant at the low-frequency limit. \(\varepsilon_s - \varepsilon_0\) \((\varepsilon_0 - \varepsilon_\infty)\) represents the dielectric relaxation strength with the time constant \(\tau_1\) \((\tau_2)\). The main relaxation time \(\tau_1\) describes the cooperative reorganization of the water molecules which is the target motion in this research. The origin the fast relaxation time \(\tau_2\) is still to be elucidated.\(^{30}\) Figure 7 shows the temperature-dependence of \(\tau_1\). In the gel phase \(\tau_1\) is reduced with the temperature, similar to the bulk water behavior.\(^{28}\) However, in the fluid phase \(\tau_1\) increases slightly. The main relaxation time of lipid solution, \(\tau_1\) is in the range of 10–11 ps. This is comparable to that of skin (10 ps at room temp.), which is largely composed of water, measured by Pickwell \textit{et al.}\(^{31}\) However, this value is somewhat higher compared to value of bulk water from Ronne \textit{et al.}\(^{28}\) \((\sim8\) ps at room temp.). This supports the restricted motion of water molecules by lipid molecules as previously discussed.

IV. CONCLUSION

This study involved an investigation of the water reorientation dynamics adsorbed on a DMPC lipid bilayer by means of THz-TDS at different temperatures. Measuring the CH\(_2\) symmetric stretching mode of the lipids with FTIR spectrometer demonstrated that below 20 °C, the lipids are in the gel
phase, and above that temperature, their phase was changed to the fluid phase. THz-TDS revealed that the hydration water ratio drastically changes from 0.3 to 0.6 during the gel-to-fluid phase transition. The absorption coefficient of the hydrated water is increased with the temperature in the gel phase and then reduced in the fluid phase. Additionally, the dielectric relaxation time of the water molecules decreases initially but increases after the phase transition. We demonstrate that THz-TDS is as an effective tool for investigating the hydration water dynamics of fully hydrated lipids. We also conclude that lipids restrict the hydration water reorientation dynamics and that this phenomenon is prominent in biologically relevant fluid phases. Further study can elucidate the number and the range of the hydration water molecules depending on the lipid phase.

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