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Article

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Water Structure at the Lipid Multibilayer Surface: Anionic Versus Cationic Head Group Effects

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ABSTRACT. Membrane water interface is a potential reaction site for many biochemical reactions. Therefore, a molecular level understanding of water structure and dynamics that strongly depend on the chemical structure of lipid is prerequisite for elucidating the role of water in biological reactions on membrane surface. Recently, we carried out femtosecond infrared pump-probe studies of water structure and dynamics at multibilayer surfaces of zwitterionic phosphatidylcholine-analog lipid (Kundu, A.; Błasiak, B.; Lim, J.; Kwak, K.; Cho, M. *J. Phys. Chem. Lett.* **2016**, *7*, 741–745). Here, to further elucidate the anionic and cationic head group

effects on water, we study vibrational dynamics of water on lipid multibilayers formed by anionic phospho-glycerol lipid molecules as well as by cationic choline-derivatized lipid molecules. We observed two significantly different vibrational lifetime components (very fast 0.5 ps and slow 1.9 ps) of the OD stretch mode of HDO molecules at the negatively charged phospho-lipid multibilayer whereas only one vibrational lifetime component (1.6 ps) was observed at the positively charged choline-derivatized lipid multibilayer. From the detailed analyses about the vibrational energy and rotational relaxations of HDO molecules in lipid multibilayers composed of anionic lipid with phosphate and cationic lipid without phosphate, the role of phosphate group in structuring water molecules at phospholipid membrane interface is revealed.

INTRODUCTION

Biological membrane separates the intracellular fluid, i.e., cytoplasm, from the outside of the cell. Typically, biological water on membrane surface has a thickness of only few nanometer, but they play a crucial role in many biochemical functions.[2,3] The orientations and functional activities of membrane-bound proteins depend on the water hydrogen-bonding network at the biological membrane.[2] Even without proteins, the structural formation and the functional activity of the biological membrane have known to be dependent on the water hydrogen-bonding network structure. Furthermore, detailed chemical structure of constituting lipid molecules affects the functional activity of the biological membrane. For instance, the interaction of DNA with membrane depends on the structure of lipid head part.[4,5] The pH at the positively charged lipid biological membrane is higher than that in the bulk whereas the pH at the negatively

charged lipid membrane is lower than that in the bulk.[6] Therefore, it is essential to know how the water structure and dynamics are affected by the chemical structure of the lipid molecules.

Most of the previous time-resolved vibrational spectroscopic studies focused on water at model biological membrane, by treating water concentration as a controllable variable.[7,8] Vibrational sum frequency generation technique has also been widely used to study water structure at the lipid monolayer (model biological membrane) for varying chemical structure of the lipid head part.[9–13] It was observed that, depending on the charge of the lipid head part, water orientation changes and is mainly governed by the electrostatic interaction. More specifically, with positively charged head part of the lipid molecules, the water is down-orientated (down orientation means the oxygen atom of water is pointing towards the lipid monolayer) whereas, in the case of negatively charged lipid monolayer, the water is up-oriented (up orientation means the oxygen atom of water is pointing away from the lipid monolayer).[12] There is both up and down orientated water molecules at the zwitterionic lipid monolayer.[9] Up orientated water is due to its interaction with negatively charged phosphate and down orientated water is due to that with positively charged choline part at the zwitterionic lipid surface.[9] Zhao et al. studied water structure and dynamics in lipid multibilayers with varying water concentration, using femtosecond mid-IR pump-probe spectroscopy.[8] Later, two-dimensional IR spectroscopy was used to further investigate the vibrational dynamics of water inside planar phospholipid multibilayer for varying concentration of cholesterol.[7] Using surface-specific vibrational pump-probe spectroscopy, Bonn et al. studied vibrational dynamics of water on lipid monolayers with different chemical structures, where they focused on the OH stretch modes of water (H_2O).[13] However, it has been known that the vibrational dynamics of water (H_2O) OH stretch modes can be complicated due to ultrafast intermolecular vibrational excitation transfer processes.[14–16]

Therefore, here we have studied how the chemical structure of lipid molecule capable of forming multibilayer morphological structures affects water hydrogen-bonding structure and dynamics, carrying out femtosecond mid-IR pump-probe measurements on the OD stretch mode of HDO molecules in the phosphate and non-phosphate lipid multibilayers.[14–16]

EXPERIMENTAL SECTION

Materials

Phospholipid is the most abundant lipid molecules in biological membrane. To address the role of phosphate in structuring water molecules near the phospholipid membrane, we need to compare structure and dynamics of water at membranes consisting of lipids with and without phosphate group. Therefore, we used an anionic lipid 1,2-dimyristoyl-sn-glycero-3-phospho-(1-rac-glycerol) (sodium salt) (DMPG) with phosphate part, and a cationic lipid, 1,2-dimyristoyl-3-trimethylammonium-propane (chloride salt) (DMTAP) without phosphate part to study water hydrogen-bonding structure and dynamics. Figure 1 shows their chemical structures. The hydrophobic tails are the same since only the effect of the head part on water hydrogen-bonding dynamics at the biological membrane is of interest. The anionic lipid (DMPG) and cationic lipid (DMTAP) were purchased as lyophilized powders from Sigma-Aldrich and used as received. Distilled water was used in preparation of isotopically diluted water (5 mol% HOD in water). The lipid multibilayer was prepared in between two 3 mm thickness CaF_2 windows as described by Zhao et al.[8] The molar ratio of water to lipid was 16. At this low hydration level, most water molecules are expected to be close to the hydrophilic head parts of the lipid multibilayers formed by DMPG and DMTAP lipid molecules, respectively. To further find quantitative evidence

supporting this, it will be necessary to carry out molecular dynamics simulations of water at the water-lipid multibilayer interface in the future, which is under current investigation in our laboratory.

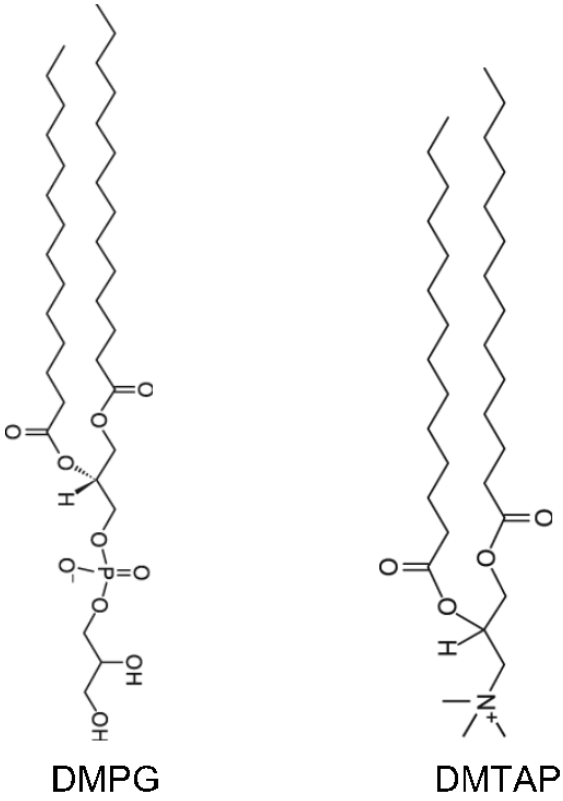

Figure 1. Chemical structure of an anionic lipid DMPG (1,2-dimyristoyl-sn-glycero-3-phospho-(12-rac-glycerol) (sodium salt)) and a cationic lipid DMTAP (1,2-dimyristoyl-3-trimethylammonium-propane (chloride salt)).

Femtosecond Mid-IR pump-probe spectroscopy

Femtosecond mid-IR pump-probe (IR PP) spectroscopy is used to obtain time- and frequency-resolved transient IR spectra of OD stretch mode of HOD molecules at these lipid multibilayers.[17–23] The experimental details of femtosecond mid-IR pump-probe spectroscopy were described previously.[21,24–26] In brief, a Ti:Sapphire regenerative amplifier system (Spectra

Physics, Spitfire Pro XP, 1 kHz, 1 mJ, 800 nm) was used as light source. The amplifier output was used for excitation of optical parametric amplifier and a difference frequency generator ($AgGaS_2$) to generate a broad-band mid-IR pulse centered at 2500 cm^{-1} with FWHM (Full width at half maximum) of 130 cm^{-1}. The mid-IR pulse was split into intense pump (~90%) and weak probe (~10%) by a beam splitter. Both the pump and probe were linearly polarized. The probe polarization was set at 45° with respect to the pump polarization. The pump beam was chopped (500 Hz) by an optical chopper system. The delay time between pump and probe pulse was controlled by a delay stage. After the sample, the probe beam after the sample passed through the motorized polarizer and entered into monochromator and detected by 64-element MCT (Mercury Cadmium Telluride) array detector. The probe polarization after the sample was selected with 0° or 90°. Every two pulses (with and without pump) were collected for each probe polarization. After subtracting the un-pumped signal from the pumped at each delay time *t*, we obtain the parallel $I_{\parallel}(t)$ and perpendicular $I_{\perp}(t)$ signals. The parallel and perpendicular signal contains both the vibrational lifetime and orientation dynamics of OD stretch mode at the lipid multibilayer. We calculate the isotropic signal by eq 1, which provide information on the vibrational lifetime,

$$P_{iso}(t) = \frac{I_{\parallel}(t) + 2I_{\perp}(t)}{3} \quad (1)$$

The orientational relaxation was estimated in the anisotropy decay (*R*) as shown in eq 2.

$$R(t) = \frac{I_{\parallel}(t) - I_{\perp}(t)}{I_{\parallel}(t) + 2I_{\perp}(t)} \quad (2)$$

The linear absorption spectra were obtained by using the VERTEX70 FTIR spectrometer (Bruker Optics) with 1 cm^{-1} resolution. The FTIR spectrum of isotopically diluted water and that

of pure water in the lipid multibilayer were measured separately. Then, the FTIR spectrum of pure H_2O in the lipid multibilayer was used as the background spectrum in obtaining the OD stretch IR band of HDO in the lipid multibilayer.

RESULTS AND DISCUSSION

Steady-state spectra

Figure 2 shows the normalized linear absorption spectra of OD stretch mode at the DMPG (red), DMTAP (black) lipid multibilayer, and in isotopically diluted bulk water (green). The peak positions of the absorption spectra are the same for all these three systems. Although the line shape of OD stretch band at the DMPG lipid multibilayer is little different from that in isotopically diluted bulk water, significant line-broadenings in both red and blue side of OD stretch band of HOD molecules at the DMTAP lipid multibilayer are observed. To compare the OD stretch IR absorption spectra of HDO in the lipid multibilayer with that in water, the corresponding difference spectra ($\Delta A = A_{\text{lipid solution}}(\omega) \quad A_{\text{water}}(\omega)$) are plotted and inserted into Figure 2 as an inset. In addition to a small frequency shift, we found that the OD stretch IR absorption spectra of HDO molecules in the DMTAP and DMPG multilayers are broader than that in water. This difference in line shapes of OD bands at DMPG and DMTAP lipid multibilayers already indicates that the equilibrium hydrogen-bonding number and hydrogen-bonding strength depend on the chemical nature of lipid head group. The OD stretch band at the DMTAP lipid multibilayer is broader than that of the DMPG multibilayer and that of bulk water, indicating more inhomogeneous broadening of OD stretch band of water molecules at the DMTAP lipid multibilayer compared to bulk water.

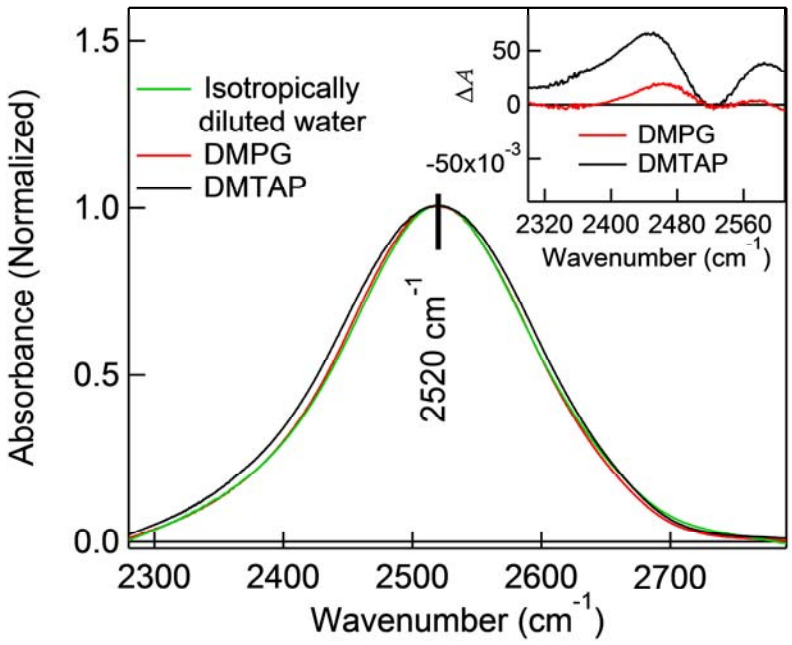

Figure 2. Background-subtracted peak normalized linear FTIR spectra of OD stretch mode of HOD probe molecules at the DMPG, DMTAP lipid multibilayer, and in isotopically diluted bulk water. The difference absorption spectra (ΔA) are shown in the inset – note that the definition of ΔA is given in the main text.

Time-resolved IR pump-probe spectra

The water dynamics at the lipid multibilayer can be studied by measuring the vibrational lifetime and rotational relaxation time of OD stretch mode, by using polarization-controlled femtosecond mid-IR pump-probe (IR PP) method. Figure 3a and b shows the isotropic IR PP spectra OD stretch modes of HOD molecules at the DMPG and DMTAP lipid multibilayers, respectively. The IR PP signals at early time delays show both positive and negative spectral features. The positive signal originates from both the ground state bleaching (v=1-0 transition) and the stimulated emission (v=1-0 transition) and the negative signal is from the excited state absorption (ESA, v=1-2 transition). Since we measure the effect of pump pulse interaction with

OD stretching vibrations of HDO on the probe beam intensity, the positive signal originates from both the ground state bleaching (GSB) and the stimulated emission (SE) and the negative signal is from the excited state absorption. The positive signal at longer time delays is due to pump-induced temperature rise. The center peak position of the bleaching signal in the DMPG lipid multibilayer is red shifted than that in the DMTAP lipid multibilayer. The peak position of the bleaching signal of OD stretch mode at the DMTAP multibilayer is the same as that of the OD stretch mode in the isotopically diluted water. The low frequency side of the positive IR PP signal of OD stretch mode of HDO in the DMPG lipid multibilayer decays faster than that in the high frequency side. In contrast, the positive IR PP signal from DMTAP multibilayer decays with the same decaying constant independent of the probe frequency.

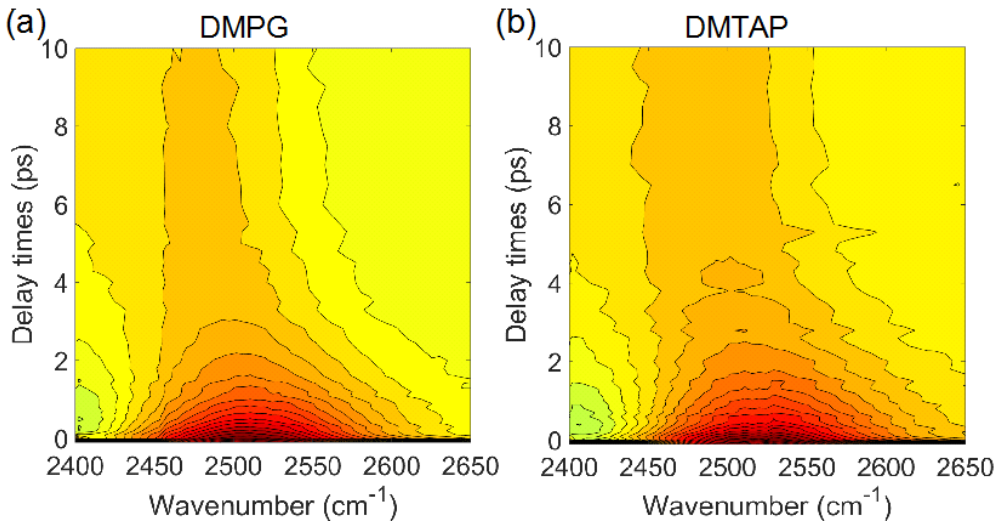

Figure 3. The isotropic IR PP signals of OD stretch of HOD molecules at the (a) anionic DMPG and (b) cationic DMTAP lipid multibilayer. The red color is the bleaching signal and stimulated emission and green color represents the excited state absorption.

We analyzed the IR PP spectra in the same way as in our previous study[1] and also as reported by Bakker et al.[27,28] In brief, the IR PP spectra of OD stretch mode at the DMPG lipid multibilayer were analyzed by taking into account two exponentially decaying components and one growing heating component. Figure 4a shows two decay components (comp1: red and comp2: blue) and a growing heating component (thermal: green). The black circles in Figure 4a are isotropic IR PP signal at 0.4 ps delay time at the DMPG lipid multibilayer. The comp1 shows only the bleaching signal whereas comp2 shows both bleaching and ESA signals. The comp1 is red shifted than that of comp2. In our previous study of water hydrogen-bonding dynamics when lipid multibilayer undergoes a temperature-induced phase transition,[1] we observed the same red shifted component and this type of the red shifted component was assigned to phosphate-bound water.[8,9,13,29] Therefore, the comp1 with sub-picosecond lifetime (0.5ps) can be safely assigned to phosphate-bound water molecules at the DMPG lipid multibilayer surface. Due to the presence of the negatively charged phosphate group at the DMPG lipid multibilayer, it creates a negative electric field along the OD stretch,[8] which red-shifts the peak position of comp1 at the DMPG lipid multibilayer. The normalized vibrational population decay of the comp1 (0.5ps) and comp2 (1.9ps) at the DMPG lipid multibilayer at the center peak wavenumber (2515 cm^{-1}) are shown in Figure 4b. The vibrational lifetime of comp1 is much faster than that of comp2 at the DMPG lipid multibilayer.

Similarly, the IR PP spectra of OD stretch mode of HOD molecules at the DMTAP lipid multibilayer system were analyzed, but here only one exponentially decaying component with 1.6ps decay constant and one growing heating component are sufficient enough to describe the time-dependent IR PP spectra (see Figure 4c). The black circles in Figure 4c are isotropic IR PP signal at 0.4 ps delay time at the DMTAP lipid multibilayer. The comp1 at the DMTAP lipid

multibilayer shows both bleaching and ESA. The normalized vibrational population decay of this comp1 at the DMTAP lipid multibilayer is shown in Figure 4d (at 2515 cm^{-1}). Clearly, the vibrational energy relaxation of OD stretch mode of HOD molecules at the DMTAP lipid multibilayer surfaces is well fitted to a single exponential function without any sub-picosecond (fast) component. This is in stark contrast with the vibrational energy relaxation pattern found at the DMPG lipid multibilayer system. Figure 4e shows the log plot of the isotropic OD stretch mode with respect to delay times at the center peak wavenumber (2515 cm^{-1}) at DMPG and DMTAP lipid multibilayer. These plot clearly shows the black line is linear with the negative slope of 1.6 and red line has little kink at early time and then it decreases linearly, which implies that the OD stretch mode at the DMPG lipid multibilayer has at least two decay components whereas the OD stretch mode at DMTAP lipid multibilayer has only one decay component. From this comparison, it is now clear that the sub-picosecond component (0.5ps) found in phosphate-containing DMPG system indeed originates from phosphate-bound water molecules, which confirms our previous explanation on the vibrational energy relaxation mechanism of phosphate-bound water.[1] Furthermore, the vibrational lifetimes of the comp2 at the DMPG lipid multibilayer and the comp1 at the DMTAP lipid multibilayer are very close to that of the OD stretch mode in isotopically diluted bulk water. These components are due to water at the very end of the lipid head part. Nonetheless, the vibrational lifetime of the comp2 in the DMPG multibilayer is slightly longer than that of the comp1 in the DMTAP multibilayer. This might be due to the extended glycol group at the DMPG lipid.

Water dynamics in various confined reverse-micelle systems has also been studied extensively.[21,29–37] It was experimentally shown that there are two very different vibrational lifetime components in such nano-confined systems. They were usually assigned to shell and

core water molecules.[21] Two different vibrational lifetime components were also observed at aligned lipid multibilayer[8] and lipid monolayer surfaces.[13] Here, we observed two distinct vibrational lifetime components in the DMPG multibilayer system, but only one vibrational lifetime component at the non-phosphate DMTAP lipid multibilayer. Thus the dominant pathway of the vibrational relaxation of the OD stretch mode of the fast comp1 at the DMPG lipid multibilayer is through the overtone band of asymmetric phosphate (PO_2^-) stretch mode[38] Note that the asymmetric stretch frequency of the PO_2^- is at 1230 cm^{-1}.[1] Therefore, the vibrational density of states (VDOS) of the overtone of the asymmetric phosphate stretch mode (2460 cm^{-1}) is close to the comp1 frequency in the case of the DMPG lipid multibilayer. This explains the 0.5 ps vibrational energy relaxation of phosphate-bound HOD molecules.

However, the predominant pathway of the vibrational energy relaxation of OD stretch mode in isotopically diluted water is through the HOD bend mode (fundamental HOD bend frequency is at 1450 cm^{-1}) and an additional excitation of lower frequency modes like librations.[39] As the vibrational lifetimes of the comp2 at DMPG multibilayer (1.9 ps) and the comp1 at the DMTAP multibilayer (1.6 ps) are close to bulk water (1.5 ps), their vibrational energy relaxation mechanisms are likely to be similar to that of the OD stretch mode in bulk water.

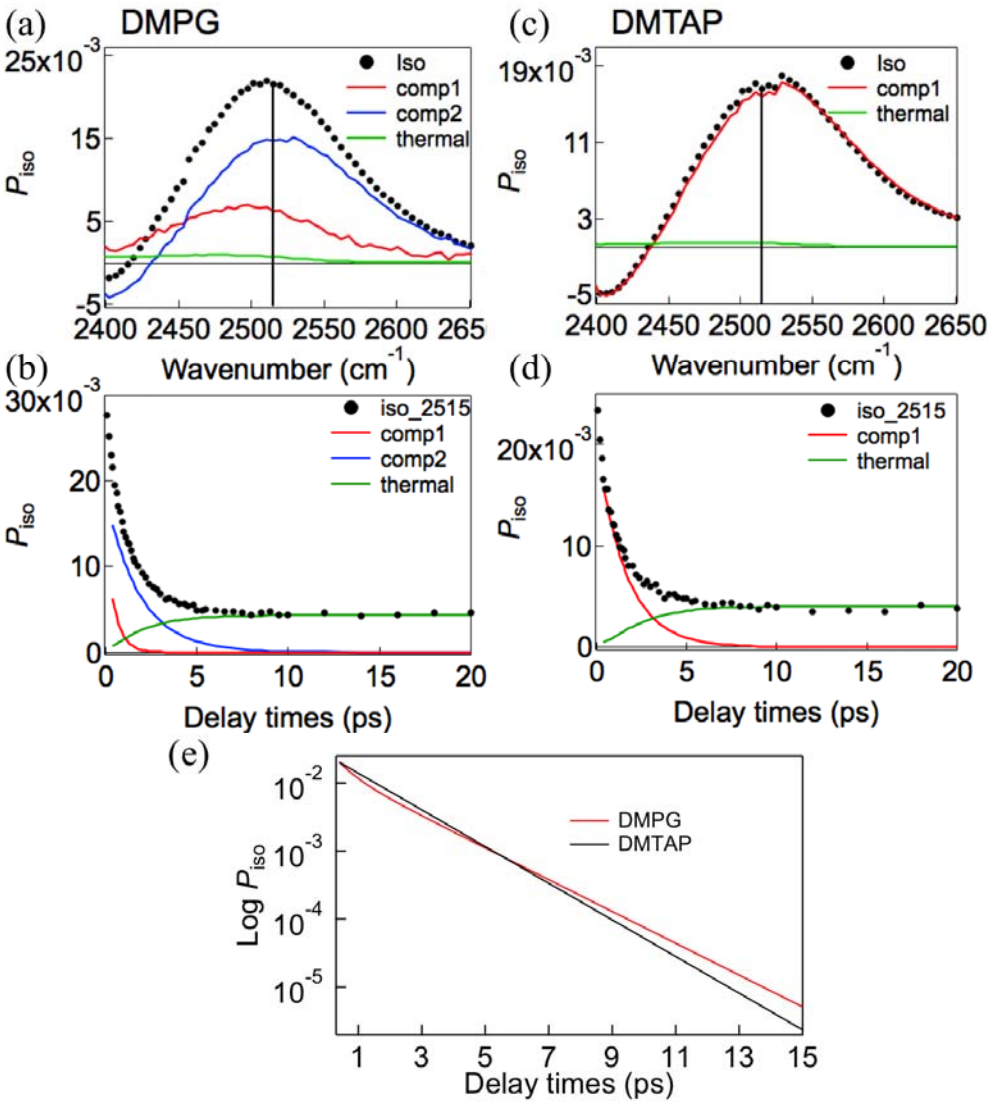

Figure 4. (a) Decomposed of IR PP spectrum at 0.4 ps (black circles) into two decay components (comp1: red and comp2: blue) and a growing heating component (thermal, green) at the DMPG lipid multibilayer (b) Experimentally measured isotropic PP data (at 2515 cm^{-1}), in the case of the anionic DMPG lipid multibilayer, are plotted together with the kinetic traces of fitting components of comp1, comp2, and thermal component. (c) Decomposed of IR PP spectrum at 0.4 ps (black circles) into one decay component (comp1) and a growing heating component (thermal, green) at the DMTAP lipid multibilayer (d) Experimentally measured isotropic PP data (at 2515 cm^{-1}) for the cataionic DMTAP lipid multibilayer are plotted together with the kinetic traces of fitting components of comp1 and thermal. (e) Logarithmic plots of the fitted isotropic signals (after subtracting thermal component from the raw data) of the OD stretch mode of HDO in DMPG and DMTAP lipid multibilayers are plotted with respect to PP delay time.

Orientational relaxation

To further investigate the water dynamics at these lipid multibilayer surfaces, we measured the IR PP anisotropy data. The orientational relaxation is related to the local environment of the vibrational probe. Since the IR probe of OD stretch of HOD, the orientational relaxation time constant provides the rotational motion of OD group in HOD. Figure 5a and b show the anisotropic decays. The rotational relaxation time of OD stretch in the DMPG lipid multibilayer was fitted with two exponentially decaying functions and that of at the DMTAP lipid multibilayer was fitted with a single exponentially decaying function. The corresponding time constants in the case of the DMPG lipid multibilayer are 0.4 ps and 4.2 ps. On the other hand, that of the DMTAP multibilayer is 1.8 ps.

To understand such notably different rotational relaxation patterns found here, it would be interesting to examine recent MD simulation studies on the orientational relaxation of water in the AOT reverse micelles. It was shown that the biexponential anisotropy decays are not from surface and bulk water molecules in the reverse miclelles.[40,41] Instead, that the fast component in the anisotropy decay is associated with the wobbling-in-a-cone motion of water whereas the slower anisotropy decay is related to complete orientational randomizations. Zhao et al. also explained their experimentally measured bi-exponential anisotropy data in the DLPC (zwitterionic lipid) lipid multibilayer systems similarly.[8] Thus, our fast anisotropic decaying component (0.4 ps) in the DMPG lipid multibilayer can be assigned to the wobbling-in-a-cone motions of water molecules. However, the slow component (4.2 ps) in the DMPG multibilayer and the single anisotropy decay component (1.8 ps) in the DMTAP multibilayer are due to the orientational randomizations. Interestingly, there are no water molecules undergoing such wobbling-in-a-cone motions in the case of the DMTAP lipid multibilayer. This is because water

molecules do not form strong electrostatic (H-bonding) interactions with the lipid head part of DMTAP. The orientational relaxation time (4.2 ps) of the slow component in the DMPG multibilayer is much slower than that in bulk (almost twice), which strongly suggest that the water molecules in the vicinity of DMPG lipid head parts are under restricted environment without many neighboring (free) water molecules assisting rotations of both phosphate- and glycol-bound water molecules. In contrast, the orientational relaxation in the DMTAP lipid multibilayer is similar to that in the bulk water, indicating that the local H-bonding structures in these two cases are quite similar.

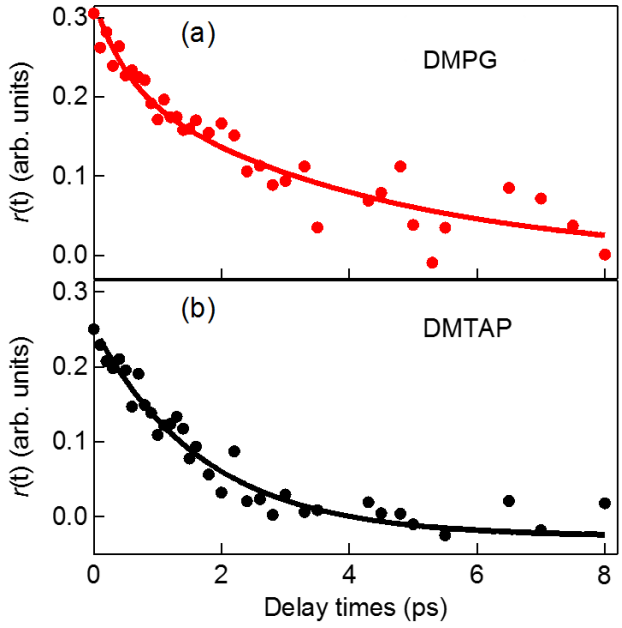

Figure 5. Anisotropy decays of OD stretch mode of HOD molecules at the lipid multibilayer. The anisotropy signals at the center probe frequency were fitted to a biexponential (at the DMPG lipid multibilayer) or signal-exponential (at the DMTAP lipid multibilayer) decay function.

CONCLUSION

In conclusion, we carried out the femtosecond mid-IR pump-probe spectroscopy and measured the water hydrogen-bonding structure and dynamics at the lipid multibilayer surfaces of lipids with phosphate and non-phosphate head groups. We observed two different vibrational lifetime components (very fast 0.5 ps and slow 1.9 ps) in the phosphate-containing DMPG lipid multibilayer and only one vibrational lifetime component in the non-phosphate DMTAP lipid multibilayer. Similarly, the water rotational dynamics was found to be sensitively dependent on the existence of phosphate group. From the mechanistic analyses of the femtosecond mid-IR pump-probe data, we show that the water structure and dynamics are strongly affected by the phosphate group. On the other hand, the positively charged of the lipid does not induce much change in the water structure and dynamics, as compared to those in bulk water. It is believed that such type of strong bound water with the presence of the phosphate part is important for many biochemical reactions as this type of water directly involved in the proton transfer at the biological membrane. We anticipate that the present experimental results and discussion will be of use in deeply understanding water's role in biochemical processes on membrane surface.

AUTHOR INFORMATION

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