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Studying Water Hydrogen-Bonding Network near the Lipid Multibilayer with Multiple IR Probes

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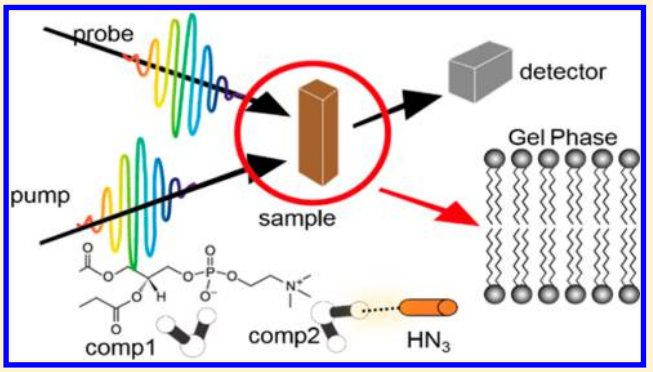
Supporting Information

ABSTRACT: A critical difference between living and nonliving is the existence of cell membranes, and hydration of membrane surface is a prerequisite for structural stability and various functions such as absorption/desorption of drugs, proteins, and ions. Therefore, a molecular level understanding of water structure and dynamics near the membrane is important to perceive the role of water in such a biologically relevant environment. In our recent paper [*J. Phys. Chem. Lett.* **2016**, *7*, 741] on the IR pump−probe study of the OD stretch mode of HDO near lipid multibilayers, we have observed two different vibrational lifetime components of OD stretch mode in the phospholipid multibilayer systems. The faster component (0.6 ps) is associated with OD groups interacting with the phosphate moiety of the lipid, while the slower component (1.9 ps) is due to choline-associated water molecules that are close to bulklike water. Here, we additionally use hydrazoic acid (HN_3) as another IR probe of which frequency is highly sensitive to its local H-bonding water density. Interestingly, we found that the vibrational lifetime of the asymmetric azido stretch mode of HN_3 in the lipid multibilayer system is similar to that in neat water, whereas its orientational relaxation is a bit slower than that in bulk water. This indicates that due to the tight packing of lipid molecules, particularly the head parts, in the gel phase, HN_3 molecules mostly stay near the choline group of lipid and interact with water molecules in the vicinity of choline groups. This suggests that membrane surface-adsorbed molecules such as hydrophilic drug molecules may interact with choline-associated water molecules, when the membrane is in the gel phase, instead of phosphate-associated water molecules.



INTRODUCTION

The importance of the biological membrane is well established in biology.[1,2] The biological membrane allows only selective species to enter the cell and is the first line of defense against entry of foreign species in a cell. The surface hydration water of the biological membrane is therefore very important and known to be quite different from bulk water.[3] Water molecules near the biological membrane maintain the structural integrity of the membrane itself. Furthermore, surface hydration water is critical in membrane functions involving molecular interaction, protein insertion, and ion transport within and across the biological membrane.[1] Hence, a deeper understanding of the water H-bonding network structure and dynamics near the biological membrane is important to understand the role of water near the biological membrane for its structural integrity and functionality.

Numerous studies using femtosecond mid-IR pump−probe spectroscopy,[4−6] vibrational sum frequency generation (VSFG),[7,8] NMR,[9] X-ray,[10] time-resolved fluorescence,[11] and molecular dynamics (MD) simulations[12] have been performed to study water H-bonding structure and dynamics in various model biological membranes. The structural information about such a membrane was also investigated using NMR and X-ray techniques.[9,10] Two-dimensional infrared spectroscopy (2DIR) study has revealed three distinct water species at the lipid/water interface of a zwitterionic lipid membrane.[13] VSFG being a surface specific technique has been used to observe orientation of water molecules at the lipid monolayer that mimics membrane.[8,14,15] The VSFG studies revealed different types of water species, H-up (oxygen atom of water pointing away from the lipid membrane) and H-down (oxygen atom pointing toward the lipid membrane) oriented water molecules on zwitterionic lipid monolayers.[8,16] The origin of H-up and H-down water species is due to presence of both negatively charged phosphate and positively charged choline groups.[8] Such orientational preference of water molecules at the lipid/water interface has also been confirmed by MD simulation.[12,16] Steady-state fluorescence and absorption spectroscopy were used to estimate probe-location-dependent polarity at the lipid/water interface in both gel and liquid crystal phases.[17] However,

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how water H-bond sensitive probe molecules behave and are distributed at the lipid/water interface has not been investigated.

Recently, tungsten hexacarbonyl [$W(CO)_6$] compound was used by Kel et al. to study the structural properties of the lipid multibilayer.[5] $W(CO)_6$, a nonpolar hydrophobic molecule, preferentially resides in the interior part of the bilayer. They observed two different vibrational lifetime components and further found that these two components are independent of the water hydration level. That indicates that the probe molecules are located in the hydrophobic region of the lipid multibilayer;[5] hence, its use for studying the water dynamics near the polar head part of the lipid may be limited.

We have used the OD stretch mode of HDO to study water H-bonding structure and dynamics near a zwitterionic lipid multibilayer using vibrational spectroscopy. A lipid multibilayer is one of the extensively used model systems mimicking a biological membrane.[5,18] Vibrational spectroscopy is an ideal method for studying heterogeneous water environment from both water's and solute's points of view in such a model system. Note that HDO is an excellent probe capable of providing direct information on water structure and dynamics from water's point of view.[19] However, it should be noted that the OD stretch mode cannot provide an answer to the question on how a given solute molecule preferentially interacting with water behaves at the lipid/water interface. In fact, the molecular mechanism of drug−membrane interaction is of particular importance in medical science.[20,21] For example, the commonly used analgesic drugs, such as aspirin (acetylsalicylic acid), was shown to interact with the headgroup of the lipid.[21] In contrast, a cosurfactant like hexanol in lipid multibilayer induces a phase transition from lamellar to hexagonal structure through altering the spontaneous curvature of the lipid monolayer.[22] Another well-known drug ibuprofen (a nonselective inhibitor of the cyclooxygenase enzyme) increases surface area of the lipid membrane.[20] However, those studies mainly focused on the distribution of drugs without investigating water structure and dynamics near them. To have a molecular level understanding of such species, e.g., solute or drug, at the lipid/water interface, here we consider a useful IR probe, hydrazoic acid (HN_3), that provides information from the dissolved solute's point of view and allows one to assess its interaction with the biological membrane, which in turn is important for the absorption/desorption of such a hydrophilic drug molecule at the membrane.

MATERIALS AND METHODS

Sample Preparation. We have used the zwitterionic lipid 1,2-dimyristyl-*sn*-glycero-3-phosphocholine (DMPC). The chemical structure of the lipid molecule is shown in Figure 1. It has a negatively charged phosphate part and a positively charged choline part. The zwitterionic lipid (DMPC) was purchased as lyophilized powders from Sigma-Aldrich and was used as received. Distilled water was used in preparation of the sample. The lipid multibilayer was prepared in between two CaF_2 windows.[5,18,23] Properly aligned lipid multibilayer is optically transparent. The molar ratio of water to lipid was kept at 16. We used isotope-diluted solution (8% HDO) to avoid complication from the vibrational excitation energy transfer between probe HDO molecules.[24,25] The HN_3 solution was prepared from 1.5 M NaN_3 solution using an ion exchange method to transform N_3^- to neutral HN_3.[26] An aqueous solution of HN_3 was then used to prepare the lipid multibilayer samples. The optical density of the azido stretch mode of the hydrazoic acid in the bulk water as well as in the lipid multibilayer solution is adjusted to be about 0.4.

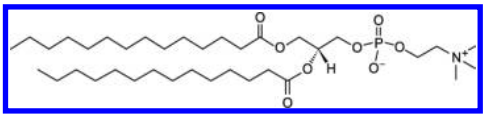

Figure 1. Chemical structure of the zwitterionic lipid 1,2-dimyristyl-*sn*-glycero-3-phosphocholine (DMPC).

FTIR Spectroscopy. The linear-IR absorption spectra were obtained by using the VERTEX70 FTIR spectrometer (Bruker Optics) with 1 cm^{-1} resolution at room temperature (298 K). The sample solutions were kept in a homemade IR cell with two CaF_2 windows to carry out FTIR and mid-IR pump−probe experiments. Before and after the femtosecond mid-IR pump−probe experiments, we measured the FTIR spectra to confirm the sample integrity.[27]

Femtosecond Mid-IR Pump−Probe Spectroscopy. Femtosecond mid-IR pump−probe (PP) spectroscopy is used to obtain vibrational lifetime and orientational relaxation time of the IR probe.[28−31] The experimental setup is described in our previous publications.[4,32,33] In brief, the laser source was a Ti:sapphire regenerative amplifier system (Spectra-Physics, Spitfire Pro XP, 1 kHz, 1 mJ, 800 nm). The amplifier output was used to pump an optical parametric amplifier (OPA) 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} to measure the OD stretch mode of HDO in bulk and near the lipid multibilayer. After completing femtosecond mid-IR PP studies of HDO systems, rotating the $AgGaS_2$ crystal, we were able to tune the mid-IR center frequency to be at 2130 cm^{-1} with fwhm of 100 cm^{-1} to measure the azido stretch mode of HN_3 in bulk and near the lipid multibilayer. Each mid-IR pulse was split into two pulses by a ZnSe beam splitter. The more intense one (∼90%) was used as pump and the weak (∼10%) was used as probe. Both the pump and probe beams were linearly polarized. The probe polarization was set at 45° with respect to the pump polarization. A small portion of the pump beam was sent to a single-element MCT (mercury−cadmium−telluride) detector and was used as a reference beam to measure the intensity fluctuation of mid-IR pulses. The delay times between pump and probe pulses was controlled using a motorized linear delay stage placed in the pump beamline. The pump beam was chopped (500 Hz) by an optical chopper system. The pump and probe beams were focused onto the sample by a 90° off-axis parabolic mirror and, after the sample, were collimated by another 90° off-axis parabolic mirror. The probe beam after the sample was passed through the motorized polarizer and entered into monochromator and detected by 64-element MCT array detector. The parallel and perpendicular components of the pump−probe signals were selected by the motorized polarizer. A polarizer was placed before the monochromator to match the intensities of the vertical and horizontal components of the probe beam onto the MCT array detector. Every two pulses (with and without pump) were collected for each probe polarization. After subtracting the unpumped signal from the pumped at each delay time t, we obtain the parallel $I_{\parallel}(t)$ and perpendicular $I_{\perp}(t)$ signals. Such polarization-controlled IR PP measurements allow us to separately measure the vibrational population relaxation from the isotropic signal defined as[31]

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

and the orientational relaxation rate from the anisotropy signal[31]

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

RESULTS AND DISCUSSION

Steady-State Spectra. Figure 2a is the background-subtracted normalized linear absorption spectra of the OD stretch mode of HDO in the DMPC lipid multibilayer (red) and in the isotopically diluted bulk water (black). We find no substantial difference between these two IR spectra, indicating that the H-bonding structure of water remains robust even in the lipid multibilayer solutions. The inset in Figure 2a shows zoomed-in spectra at the peak position. It reveals that the OD stretch IR spectrum of HDO in the DMPC multibilayer is slightly red-shifted in comparison to that in bulk water. This is due to the negatively charged phosphate moiety that creates a strong electric field along the OD bond of HDO molecules near the lipid multibilayer−water interface. Similarly, the OD stretch mode of HDO in fluoride salt solution was found to be red-shifted with respect to bulk water because the negatively charged F^- ions directly interacting with OD group induce a stronger solvatochromic vibrational frequency shift of the OD stretch mode as compared to those OD groups H-bonded with other water molecules.[34]

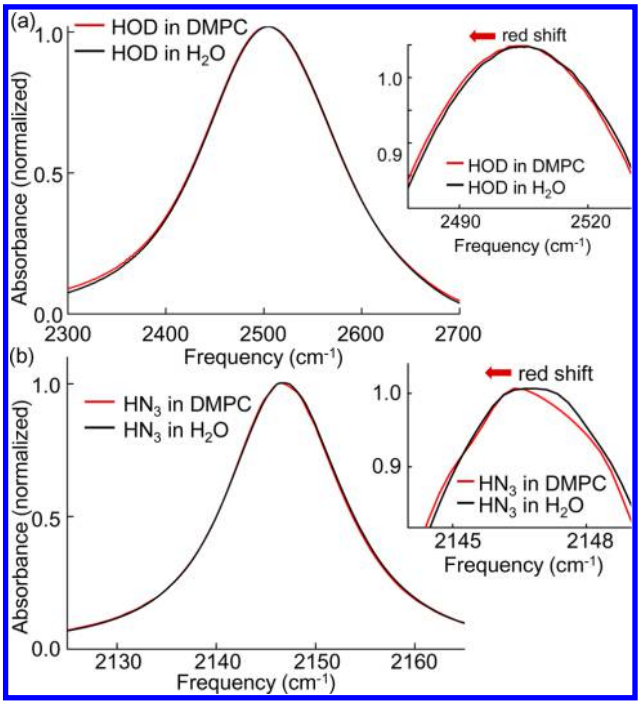

Figure 2. Background-subtracted normalized linear-IR absorption spectra of the OD stretch mode of HDO molecules in the lipid multibilayer (red) and that in the isotopically diluted water (black). (b) Background-subtracted normalized linear-IR absorption spectra of the asymmetric azido stretch mode of HN_3 molecules in the lipid multibilayer (red) and in bulk water (black). The insets of (a) and (b) are the zoomed-in spectra at the peak maxima.

The background-subtracted normalized IR spectra of azido stretch mode of HN_3 molecule in the DMPC lipid multibilayer (red) and in the bulk water (black) are shown in Figure 2b. The azido band near the lipid multibilayer exhibits a negligibly small red-shift with respect to bulk water (see the inset in Figure 2b. To examine the effect of negatively charged phosphate group in the DMPC lipid molecules on the asymmetric azido stretch mode of HN_3 in the vicinity of the polar headgroup of DMPC, we measured the azido stretch IR spectra of HN_3 in 4 M sodium monophosphate solution and found that the azido band undergoes a blue-shift and not a red-shift (see Figure S1 in the Supporting Information) with respect to bulk water. This indicates that HN_3 molecules in the lipid multibilayer are exposed to a bulklike water environment, whereas in phosphate salt solutions, water−water H-bonding is substantially broken and enhancing water−HN_3 H-bond populations to compensate for the broken H-bond of water in salt solutions. Therefore, it is believed that a small red-shift of the azido stretch mode of HN_3 near the lipid multibilayer can be explained in terms of confinement effects, as discussed before for HN_3 molecules dissolved in water that is confined by reverse micelle systems.[35]

Time-Resolved IR PP Spectra of OD Stretch Mode. To quantify the water H-bonding dynamics in the lipid multibilayer, we measured the vibrational lifetime of the OD stretch mode of HDO molecules with the femtosecond mid-IR pump−probe spectroscopy. The experimental results on vibrational spectroscopy of HDO in the DMPC multibilayer were presented in our previous report.[4] However, the main results and findings are briefly summarized here for the sake of direct comparisons with the present experimental results with HN_3 probe (next section). The isotropic IR PP spectra of OD stretch mode of HDO in the isotopically diluted water and those in the DMPC lipid multibilayer are shown in Figures S2a and S2b, respectively. The positive signal is due to ground state bleaching ($v = 0$ to $v = 1$ transition, GSB) and the stimulated emission ($v = 1$ to $v = 0$ transition, SE), and the negative signal is due to the excited state absorption ($v = 1$ to $v = 2$ transition, ESA).The positive signal at long delay times (25 ps) in both systems is dictated by the pump-induced temperature rise of the sample.

The kinetic traces of the isotropic IR PP signal of the OD stretch mode in the isotopically diluted water at different probe frequencies are shown in the Supporting Information (Figure S3a). They are independent of the probe frequency at short times up to ∼1.8 ps. However, the decaying pattern becomes dependent on probe frequency at longer delay times due to the in-growing heating contribution to the isotropic IR PP signals. We fitted the isotropic IR PP spectra of the OD stretch of HDO in isotopically diluted water with one exponentially decaying component and one in-growing heating component.[30] The decay component (comp1) and the in-growing heating component (thermal) are shown in red and green, respectively, in Figure 3a.

The clear spectral shape of the thermal component is shown in Figure S3b. The black circles in Figure 3a are the isotropic IR PP spectrum at time delay of 0.4 ps. The corresponding kinetic trace of the comp1 and the thermal component are shown in red and green, respectively, in Figure 3b. The black circles in Figure 3b are the isotropic decay data at 2500 cm^{-1} (see the black vertical line in Figure 3a). The vibrational lifetime of the comp1 is 1.7 ps, which agrees well with the previously reported values.[30,36]

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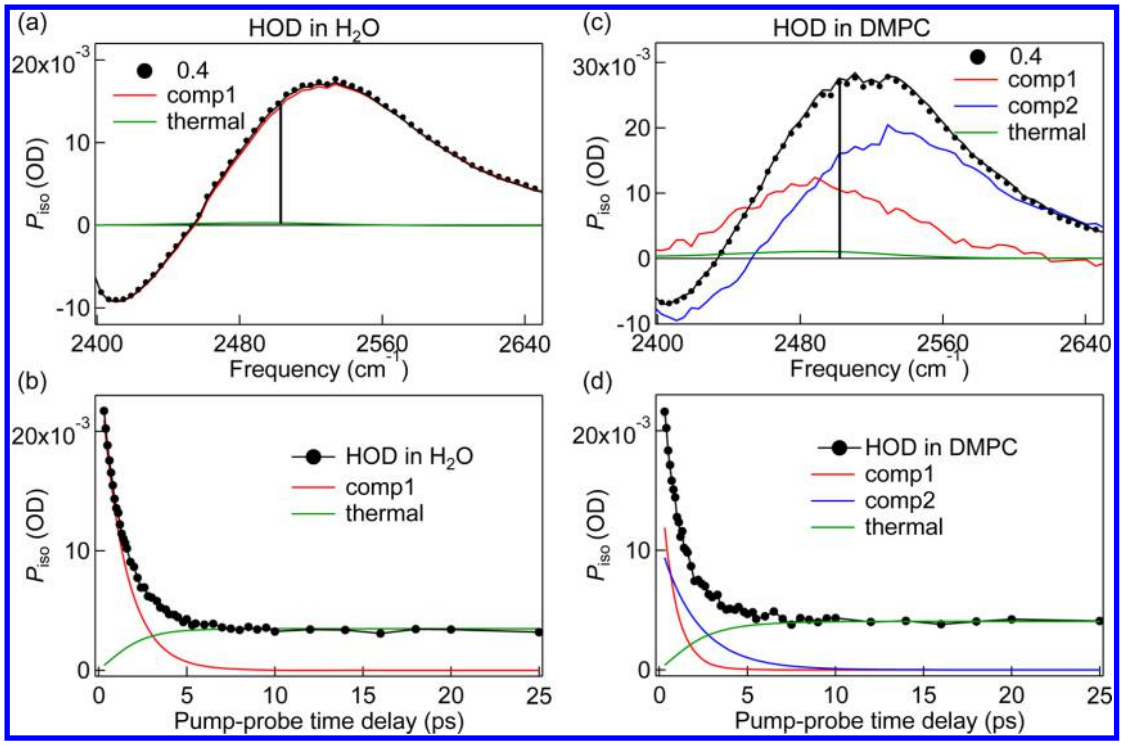

Figure 3. (a) Isotropic IR PP signal (black circles, at 0.4 ps) of the isotopically diluted water. The spectrum can be decomposed into one decaying (red) and one in-growing components (green). (b) The black circles are the temporal profile of the IR PP signal at 2500 cm^{-1}, and the red and green lines are the kinetic traces of these two components. (c) The isotropic signal (black circles, at 0.4 ps) of HDO in the DMPC lipid multibilayer can be decomposed into two decaying (red: comp1; blue: comp2) and one in-growing components (green). (d) Red, blue, and green lines are the kinetic traces of these components.

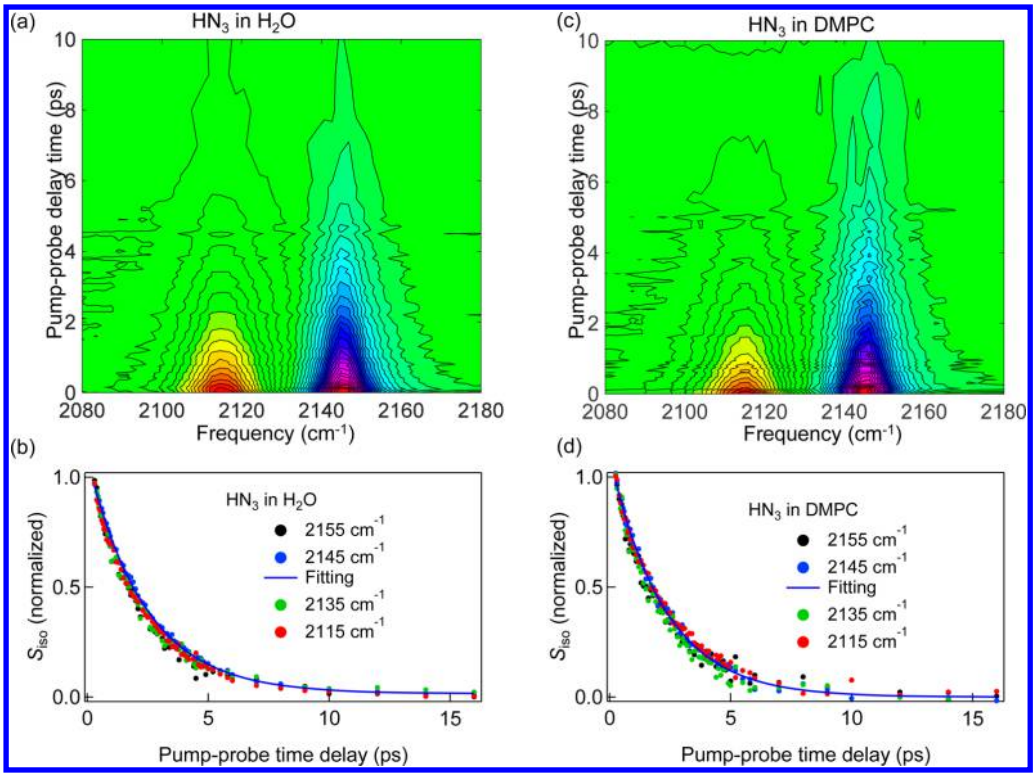

Figure 4. (a) and (c) are the isotropic IR PP spectra of the asymmetric azido stretch mode of HN_3 in bulk water and in the DMPC multibilayer solution, respectively. The normalized kinetic traces of HN_3 in water (b) and in DMPC multibilayer (d) at different probe frequencies.

The kinetic traces of the isotropic IR PP signal of the OD stretch mode in the DMPC lipid multibilayer at the red and blue sides are shown in Figure S4a. The kinetic trace on the red (low frequency) side is faster than that on the blue (high

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frequency) side. This indicates that there are at least two different vibrational lifetime components. Hence, the isotropic IR PP spectra were fitted with two decay components and one in-growing heating component.[4] The fitted data (solid lines) are also plotted in Figure S2b. The eigen spectra of these two decay components (comp1 and comp2) and one in-growing heating component (thermal) are shown in Figure 3c in red, blue, and green color, respectively. The IR PP spectrum of the heating (thermal) component is also shown in Figure S4b. The peak position of the comp1 is red-shifted with respect to comp2. The comp1 is assigned to the phosphate-associated water, and comp2 is assigned to the choline-associated water as reported in our recent study.[4] The negatively charged phosphate creates an electric field along the OD bond, which causes a frequency red-shift of the comp1 spectrum. The kinetic traces of the comp1, comp2, and thermal components are separately plotted in Figure 3d, and the black circles are the total isotropic IR PP data at 2500 cm^{-1} (see the black vertical line in Figure 3c). The vibrational lifetime of the comp1 is 0.6 ps, and that of the comp2 is 1.9 ps.

Interaction of HN_3 Molecules with Water near the Lipid Multibilayer. The isotropic IR PP spectra of the azido stretch mode of HN_3 molecules in the bulk water and in the DMPC lipid multibilayer at different PP delay times are shown in Figures 4a and 4b, respectively. The average vibrational lifetimes in these two systems are obtained after correcting the thermal component. The vibrational population decay function $P_{iso}(t)$, should approach zero at sufficiently long delay times, but there is a finite signal even at very long times (Figure S5c). This finite asymptotic value results from the effect of pump-induced heating of water,[37] since the azido stretch IR band spectrally overlaps with the water combination band (see Figure S6). To clarify the origin of the local heating contribution to our IR PP signals, we carried out time-resolved IR PP study of poly(ethylene glycol) methyl ether azide in dichlormethene (water free) solution. We did not observed any heating component contributing to the isotropic IR PP signal.[38] Therefore, it is naturally to conclude that the long-time component in the isotropic IR PP spectra of the HN_3 molecules in bulk water and DMPC multibilayer is clearly due to the pump-induced heating of water. Now, the transient heating contribution should be subtracted out from the raw IR PP data,[32] where the heating component is modeled as a rising function:

$$h(t) = \left(1 - \exp\left(-\frac{1}{T_{heat}}\right)\right) \quad (3)$$

Here, the time constant T_{heat} represents the local heating rate, and this value was taken from the literature (0.9 ps).[39] We found that the total IR PP signal at long delay times is completely dictated by the heating component. Therefore, the heating component-free IR PP signal can be obtained as

$$S(\omega, t) = P_{iso}(\omega, t) - h(t)P_{iso}(\omega, \infty) \quad (4)$$

The isotropic signals before and after the heating correction are shown in Figures S5a and S5b, respectively. The normalized population decays of the azido stretch mode of HN_3 in the bulk water and in the DMPC lipid multibilayer are shown in Figures 4b and 4d, respectively. The kinetic traces decay on the same time scale regardless of probe frequency. They can be fitted with a single-exponential function. The vibrational lifetime of HN_3 in bulk water agrees well with previously reported value (2.3 ps).[40] The vibrational lifetime of HN_3 in the DMPC lipid multibilayer (2.2 ps) is similar to that in bulk water. Recently, we reported that the vibrational lifetime of the asymmetric azido stretch mode of HN_3 strongly depends on its H-bonding environment.[38] Thus, the observation that the vibrational lifetime of the azido stretch mode near the DMPC multibilayer is similar to that in bulk water indicates that local H-bonding environment around each HN_3 molecule even in the lipid multibilayer system is quite similar to that in bulk water. This is surprising because the zwitterionic headgroup of the lipid molecule with a negatively charged phosphate and a positively charged choline group can significantly restructure water H-bonding network at the DMPC lipid multibilayer surface as was inferred from the vibrational lifetime study of the OD stretch mode of HDO in the same system.

Recently, Barrett et al. studied the interaction of a drug molecule, aspirin, with aligned lipid membrane on a glass substrate, using X-ray diffraction. They found that aspirin molecules mostly reside in the headgroup region of the lipid bilayer.[21] However, X-ray diffraction study does not reveal water structure and dynamics near the drug aspirin. Here, we have used a water-sensitive probe HN_3. The observation that the vibrational lifetime of azido stretch mode in the lipid multibilayer system is similar to that in bulk water suggests that the solute (HN_3) preferentially interacts with water molecules associated with choline groups, not with those phosphate-associated water. At temperatures below the phase transition temperature of the DMPC multibilayer (307 K), because of tight packing of the lipid headgroups in the gel phase,[4] the HN_3 molecules are likely to be close to the positively charged choline group. Therefore, we anticipate that biomolecules or chemicals such as hydrophilic drugs would interact with water molecules in the vicinity of choline groups of lipid bilayer when it is in the gel phase. However, since this conclusion is based on the behavior of highly water-sensitive hydrophilic IR probe, HN_3, in the present work, it would be necessary to examine how the size, shape, polarity, and hydrophobicity of a given IR probe affect water structure in the vicinity of lipid bilayer and even its structure.

Orientational Relaxation of HN_3. The orientational relaxation time of the azido stretch mode of HN_3 near the lipid multibilayer can be estimated from the anisotropic signal at the center frequency (2145 cm^{-1}) of the asymmetric azido stretch IR band. The anisotropy decay profiles for HN_3 molecules in the bulk water (black) and in the lipid multibilayer (red) are plotted in Figure 5. In the case of bulk water solution, the anisotropy signal decays exponentially with time constant of

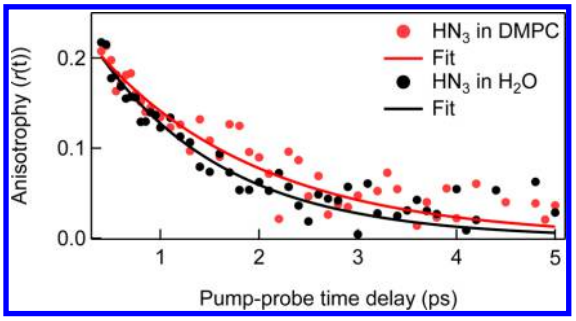

Figure 5. Anisotropic decays of the azido stretch mode of HN_3 molecules in the DMPC lipid multibilayer (red) and in bulk water (black). Solid lines are the fitted curves.

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1.2 ± 0.1 ps, which agrees well with previously reported value.[29] The anisotropy signal of HN_3 in the lipid multibilayer can also be well fitted with a single-exponential function, and the orientational relaxation time constant is found to be 1.8 ± 0.2 ps.

Hynes et al. reported that the orientational relaxation of water involves concerted breaking and re-forming of H-bonds of the water molecule in a bifurcated H-bond configuration with two other water molecules (incoming and outgoing), and their simulated data agree well with the experimentally measured orientational relaxation of OD in bulk water.[41,42] Similarly, if there is a factor hindering any breaking and/or making of H-bonds of HN_3 molecule with neighboring water molecules, the orientational relaxation of HN_3 would be slowed down. Recently, we have shown that the orientational relaxation of HN_3 molecules becomes slow upon increasing concentrations of various osmolytes.[29] The fact that the orientational lifetime of HN_3 molecules in DMPC lipid multibilayer is slower than that in the bulk water indicates that HN_3 molecules are located near the headgroup of the lipid molecules in the lipid multibilayer. An interesting study by the Ziegler group was performed to show that NO_2 is a useful IR probe for studying water and lipid bilayer structures.[43] However, unlike NO_2, our IR probe HN_3 molecules are preferentially located on the surface of lipid bilayer so that its vibrational properties report highly local and selective water structure and provide information on approximate number density of water molecules at the choline part of the headgroup.

CONCLUSION

In this paper, we report femtosecond mid-IR pump−probe spectroscopy and FTIR measurements on the azido stretch mode of HN_3 molecule in the zwitterionic lipid multibilayer system, and the results were compared with those of the OD stretch mode of HDO molecules in the DMPC lipid multibilayer and bulk water that were reported before.[4] Previously, we observed two distinct vibrational lifetime components of the OD stretch mode of HDO molecule near the lipid multibilayer due to presence of two different subpopulations of water molecules that interact with either negatively charged phosphate groups or positively charged choline groups of the lipid DMPC molecules. In contrast, we observed only one vibrational lifetime component of the asymmetric azido stretch mode of HN_3 probe molecules in the lipid multibilayer system as well as in the bulk water. The corresponding lifetimes are quantitatively similar in both cases, indicating that the HN_3 molecules even in the vicinity of bilayer surface form a strong H-bonding interaction with water and are preferentially located close to the choline group due to the compact structure around the phosphate groups in the gel-phase multibilayers at room temperature. Therefore, on the basis of the present work, we anticipate that hydrophilic molecules, e.g., water-soluble drugs, prefer to interact with the choline-associated water molecules when the biological membrane is in the gel phase.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpca.6b12152.

(1) Linear normalized FTIR spectra of the azido stretch mode of HN_3 in the sodium monophosphate solution, (2) isotropic IR PP spectra in the isotopically diluted water and in the DMPC lipid multibilayer, (3) kinetic traces of the isotropic IR PP signals at different wavenumbers of the OD stretch mode in the isotopically diluted water and the ingrowing thermal component, (4) kinetic traces of the isotropic IR PP signals at different wavenumbers of the OD stretch mode near the zwitterionic lipid multibilayer and the ingrowing thermal component, (5) IR pump−probe (PP) spectra and the kinetic traces of the azido stretch mode HN_3 before and after heating correction, and (6) FTIR spectra of water combination mode and azido stretch mode (PDF)

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Notes

The authors declare no competing financial interest.

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