Hydration and vibrational dynamics of betaine (N,N,N-trimethylglycine)

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(Received 15 January 2015; accepted 23 April 2015; published online 8 May 2015)

Zwitterions are naturally occurring molecules that have a positive and a negative charge group in its structure and are of great importance in many areas of science. Here, the vibrational and hydration dynamics of the zwitterionic system betaine (N,N,N-trimethylglycine) is reported. The linear infrared spectrum of aqueous betaine exhibits an asymmetric band in the 1550-1700 cm⁻¹ region of the spectrum. This band is attributed to the carboxylate asymmetric stretch of betaine. The potential of mean force computed from *ab initio* molecular dynamic simulations confirms that the two observed transitions of the linear spectrum are related to two different betaine conformers present in solution. A model of the experimental data using non-linear response theory agrees very well with a vibrational model comprising of two vibrational transitions. In addition, our modeling shows that spectral parameters such as the slope of the zeroth contour plot and central line slope are both sensitive to the presence of overlapping transitions. The vibrational dynamics of the system reveals an ultrafast decay of the vibrational population relaxation as well as the correlation of frequency-frequency correlation function (FFCF). A decay of ~0.5 ps is observed for the FFCF correlation time and is attributed to the frequency fluctuations caused by the motions of water molecules in the solvation shell. The comparison of the experimental observations with simulations of the FFCF from *ab initio* molecular dynamics and a density functional theory frequency map shows a very good agreement corroborating the correct characterization and assignment of the derived parameters. © 2015 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4919795>]

I. INTRODUCTION

It has been postulated that molecules can induce either order or disorder in the water network when present in water. Solutes that induce order are defined as kosmotropes, while those that induced disorder are chaotropes.¹ The chaotropes are solutes that strongly interact with water, through, for example, charged groups, and consequently their aqueous solutions have a higher viscosity than the pure water.² On the other hand, kosmotropes have more repulsive interactions with water, such as hydrophobic hydration, which can result in a viscosity lower than pure water.² Interestingly, some “structure-maker” solutes, such as betaine,³ have both hydrophobic and hydrophilic moieties in their structure, raising questions about the molecular mechanism of these kosmotropes. To understand how these “structure-makers” interact with water, a detailed characterization of the solvation of these solutes is required. Here, our study focuses on the characterization of the hydration dynamics of betaine (N,N,N-trimethylglycine).

Betaine is a small zwitterion (Figure 1) consisting of a negatively charged carboxylate group and a positively charged tetramethyl-ammonium group linked by just a methylene group, resulting in a comparatively small separation between the two charged groups. The small distance between the two groups also produces a structurally heterogeneous solvation shell which is highly influenced by both the hydrophilicity

of the carboxylate group and the hydrophobicity of the methyl groups from the ammonium moiety. In other words, the alkyl-ammonium moiety promotes the orientation of the water molecules such that the water dipole points towards the betaine nitrogen atom but keeps the water hydrogens away such that they are hydrogen-bonded with other waters. On the other hand, the carboxylate group strongly binds to water molecules through their hydrogen atoms. As a consequence, it is expected that the hydration dynamics of both the hydrophilic and hydrophobic moieties of betaine will impact the overall hydration dynamics of the system. Thus, the unusual structure of the betaine solvation shell opens a new question: Which of the two solvation shells (corresponding to the positively charged hydrophobic region or the negatively charged hydrophilic site of betaine) dominates the structure and dynamics in the aqueous solution of this amphiphilic zwitterion?

The solvation structure and dynamics of betaine in water remain poorly characterized. There are only a few experimental and theoretical studies in the literature.³⁻¹⁴ In particular, the study of the structure and dynamics of betaine in aqueous solution has been limited to dielectric relaxation^{6,7} and Raman experiments,^{3,5} and molecular dynamics simulations.^{10,12,13} It has been inferred from the Raman study that betaine adopts a conformation in which one of the oxygen atoms of the carboxylate points towards the nitrogen atom of the ammonium groups acquiring a similar structure to that seen in the anhydrous betaine single crystal.³ Moreover, the same study showed that the water molecules interact

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FIG. 1. Structure of betaine. The grey and pink planes define the dihedral angle (Φ) as describe in the text.

very weakly with the ammonium portion of the zwitterion.³ In addition, dielectric relaxation studies have shown that betaine has a rotational relaxation of ~ 40 ps and that the number of water molecules in the solvation shell is 9, where only two of these water molecules are tightly bound to betaine.^{6,7} Molecular dynamics studies performed either by quantum-mechanics/molecular mechanics (QM/MM)^{10,12} or MM/MM^{11,13,14} have proposed that betaine in solution has the same structure as that inferred from the Raman experiment and does not aggregate because it is strongly solvated by water. Moreover, it has been predicted that the water molecules close to the betaine carboxylate group have a resident time much longer than for other carboxylate groups indicating a strong binding between betaine and water.¹³ However, the overall effect of betaine on the structure of water is to strengthen the water network as seen from the self-diffusion coefficient of water.¹⁵ Thus, the theoretical works are in agreement with betaine being a “structure-maker.”

In this study, non-linear infrared (IR) spectroscopies in conjunction with *ab initio* molecular dynamics (AIMD) simulations have been carried out in order to gain insight into the hydration dynamics of aqueous betaine. The vibrational spectroscopic studies focus on the asymmetric stretch mode of the carboxylate group. It has been shown previously that this particular oxygen-carbon-oxygen (OCO) mode is a good sensor of the local solvation environment and its ultrafast dynamics.^{16–19} Moreover, the investigation of the carboxylate vibrational modes in conjunction with ultrafast IR non-linear spectroscopies provides direct insight into the dynamics and composition of the solvation shell, which are not readily obtained by other conventional techniques, such as Raman scattering. This advantage added to the sub-picosecond time resolution makes non-linear ultrafast spectroscopies the preferred tool to elucidate the dynamics and mechanism of processes occurring in water.^{20–25}

In this work, ultrafast IR pump-probe, two dimensional infrared (2D IR), and linear absorption IR spectroscopies are used to characterize the vibrational dynamics of the carboxylate asymmetric stretch of betaine. Furthermore, to

obtain the atomistic model giving rise to the observed vibrational dynamics of betaine, the conformational and hydration dynamics of betaine are studied using *ab initio* molecular dynamics. Finally, the non-linear vibrational spectroscopy of betaine is also modeled with the non-linear response theory.

The rest of the paper is organized as follows. Section II discusses the experimental and computational methodologies used in this work. The results are presented in Sec. III, followed by a discussion in Sec. IV. Conclusions are presented in Sec. V.

II. EXPERIMENTAL AND THEORETICAL METHODS

A. Sample preparation

Betaine ($\text{C}_5\text{H}_{11}\text{NO}_2$, >99%) and deuterium oxide (D_2O , 100.0 at. % D) were obtained from Acros Organics. Ethylene glycol ($\text{C}_2\text{H}_6\text{O}_2$ anhydrous, >99.8%) was purchased from Sigma Aldrich. All chemicals were used without further purification. The solute was dissolved in D_2O to obtain a concentration of 0.10 M. The sample was held in between two CaF_2 windows of 2 mm separated by a spacer of 25 μm in the FTIR experiment and of 6 μm in the 2D IR and pump-probe experiments. Unless otherwise noted, all the experiments were performed at room temperature (20 °C).

B. Linear IR spectroscopy

Linear FTIR spectra were obtained with a Bruker Tensor 27 spectrometer having 0.5 cm^{-1} resolution. The FTIR studies were performed with a temperature controlled liquid cell (Harrick Scientific). The temperature was regulated with a Hart Scientific temperature controller and a Pharmacia Biotech Multi Temp III Recirculator/Chiller circulating bath.

C. 2D IR spectroscopy

A detailed description of the 2D IR photon echo experiments can be found in a previous publication.²⁶ Briefly, a home-made Ti:sapphire amplifier coupled to a homemade optical parametric amplifier (OPA) with a difference frequency generator produces nearly Fourier transform limited IR pulses. The IR pulses have an 80 fs duration and are centered at 1625 cm^{-1} with 400 nJ energy. These IR pulses are split into three replicas (wave vectors: $\mathbf{k}_1$, $\mathbf{k}_2$, and $\mathbf{k}_3$) and focused at the sample using the box configuration geometry. The polarization of each of the pulse is set by a polarizer before the sample.²⁷ The photon echo signal in the phase matching direction ($-\mathbf{k}_1 + \mathbf{k}_2 + \mathbf{k}_3$) is heterodyned with a fourth IR pulse (LO) and detected with a liquid nitrogen cooled 64 element MCT array detector after dispersion in a monochromator (50 grooves/mm). In all the experiments, the LO pulses preceded the signal field by ~ 1 ps. To obtain the corresponding two-dimensional spectra, two time sequences of pulses are used. The rephasing (non-rephasing) echo signal is measured when the pulses with the wave vector $\mathbf{k}_1(\mathbf{k}_2)$ arrived at the sample before those with wave vector $\mathbf{k}_2(\mathbf{k}_1)$. The coherence and waiting time intervals consist of a total time interval of 3 ps with a 2 fs step, and of 0 ps–1.2 ps in steps of 100 fs, 250 fs step from 1.5 to 2 ps, and 500 fs until 2.5 ps, respectively.

D. Gas phase *ab initio* calculations

The potential energy scan of the OCCN dihedral angle was carried out at both the MP2 as well as the density functional theory (DFT), specifically BLYP,^{28,29} level of theory (with the Grimme empirical dispersion correction³⁰ for the latter case) with the TZV2P basis set.³¹ The calculations of the vibrational frequencies for the two betaine conformers were performed at the DFT (B3LYP) level of theory with the 6-311++G** basis set and a polarizable continuum model for the solvent. All these calculations were performed using the GAUSSIAN 09 software package.³²

E. Molecular dynamics simulations and free energy calculations

The free energy profile of the OCCN dihedral angle of betaine in the condensed phase was determined using umbrella sampling (US)³³ simulations with a classical force-field as well as *ab initio* based methods.

1. AIMD simulations

Born-Oppenheimer molecular dynamics simulations were carried out using the BLYP functional with the empirical dispersion correction of Grimme and the TZV2P basis set with Goedecker-Teter-Hutter pseudopotentials³⁴ for the inner core electrons. This functional along with the empirical dispersion correction was chosen because it reproduces the structural and dynamical features of liquid water.³⁵ The system consists of a single betaine molecule solvated by 82 waters in a cubic box of length 14 Å with periodic boundary conditions at a temperature of 300 K (using a Nose Hoover thermostat with a 100 fs temperature damping constant). Note that the system consisting of 82 waters was chosen because it replicated the potential of mean force calculated for a larger system using molecular dynamics with a classical force field (see below). A starting configuration for the umbrella sampling AIMD (US-AIMD) simulations was generated by carrying out classical simulations of this system using the general amber force-field.³⁶ One of the two OCCN angles was arbitrarily chosen for the US-AIMD simulations, and ten US windows, from 0° to 90°, were used with a harmonic US potential of the form,

$$V_{US} = k(\Phi_{OCCN} - \Phi_{OCCN}^0)^2,$$

with a force constant (k) of 31.5 kcal mol⁻¹ rad⁻². Each window was equilibrated for 2 ps followed by a 10 ps production run with a 0.5 fs time step. The weighted histogram analysis method (WHAM)³⁷ was used to obtain the free energy profile from the probability distribution of the dihedral angle from the various US windows.

In addition to the US-AIMD simulations, canonical simulations (in the NVT ensemble) were also carried out. The starting configurations for ten such NVT simulations were generated from the umbrella sampling windows. Each simulation was equilibrated for 1 ps and then a 10 ps production run was carried out with a time step of 0.5 fs.

2. Classical force-field

The free energy profile of the dihedral angle of betaine in water was also determined by performing umbrella sampling simulations with the general amber force-field³⁸ on the same system but with a force constant of 50 kcal mol⁻¹ rad⁻² and 18 windows between 0° and 90°. Each US window simulation was carried out for 1 ns after an initial equilibration of 500 ps, with a time step of 1 fs. Canonical simulations (in the NVT ensemble) were also carried out using the system described above. In order to test that the system consisting of 82 waters was sufficiently large, a second set of umbrella sampling simulations with a larger system consisting of 1 betaine and 2475 water molecules with a box length of 42.5 Å was also carried.

All AIMD simulations were carried out using the CP2K³⁶ software whereas the classical simulations were carried out using the GROMACS³⁹ software package.

F. Theoretical frequency-frequency correlation function (FFCF)

A DFT map developed for the acetate ion⁴⁰ was used to compute the frequency fluctuations of the carboxylate asymmetric stretch mode of betaine from the AIMD simulations. The DFT map relates the electric fields and their gradient produced by the water molecules (represented by 3 point charges, one at the O site and the other two at the H sites of the water molecule) at the C, O1, and O2 atoms of betaine with the asymmetric stretch frequency. This map uses 33 electrostatic parameters, five of which correspond to components of electric fields at these atoms and the remainder to electric field gradients at the same points. The electrostatic parameters were derived by linear least square fitting of vibrational frequencies computed by DFT with the electric field and its gradient of the water molecules, each represented by 3 point charges. It has been previously shown that the fluctuations of the frequencies and the linear infrared spectrum estimated with this DFT frequency map methodology produced a good agreement with experimental data.¹⁹

Using this map on the trajectories from the canonical AIMD simulations, the frequency ($\omega(t)$) of the asymmetric stretch mode of the carboxylate group of betaine at a given time step was calculated, which in turn was used to determine the frequency-frequency time auto-correlation function, $\langle \delta\omega(t)\delta\omega(0) \rangle$, where $\delta\omega(t) = \omega(t) - \langle \omega \rangle$.

In the computation of the FFCF, ten random starting configurations were used to carry out ten independent canonical NVT simulations with a 10 ps production time. For each of these trajectories, a FFCF was calculated. Thus, the computed FFCF is an average of 10 different and independent FFCFs.

III. RESULTS

A. Experiment

The FTIR spectrum of 0.1 M solution of betaine shows an absorption peak on top of the broad combinational band of deuterated water in the IR region between 1550 and 1700 cm⁻¹ (Figure 2). The narrow absorption band located

FIG. 2. FTIR spectra of 0.1 M solution of betaine in D_2O with solvent background subtracted. The top panels show the comparison between the experimental absorption line shape (black filled squares) and the fit with one (red line) and two (blue line) Voigt profiles, and the residual of the fits. The bottom panels display the FTIR at two different temperatures where the black filled squares are the experimental data points, and the blue and black lines are the least square fit and the residuals, respectively. The dashed red and dotted green lines are the individual Voigt profiles obtained from the fit. The insets in the top and bottom panels correspond to the betaine spectrum without subtraction of the background.

at $\sim 1625\text{ cm}^{-1}$ corresponds to the asymmetric stretch of the betaine carboxylate group and is in agreement with previous assignments of the vibrational modes of betaine.³ The carboxylate asymmetric stretch band of aqueous betaine is slightly asymmetric and can be modeled reasonably well with a Voigt profile line shape function. However, this band is better described by two Voigt profiles as shown by the residuals (top panels of Figure 2).⁴¹ In addition, it will be shown later in this study that the presence of two transitions is also consistent with the betaine 2D IR spectra and their time evolution. The least squares fit of the peak with two Voigt functions (Figure 2) yields low and high frequency peaks located at 1625 cm^{-1} and 1633 cm^{-1} , respectively, with a ratio of peak areas between the low and the high frequency peaks of 0.2 and the full width at half maximum for both peaks is $\sim 25\text{ cm}^{-1}$. Interestingly, the Voigt fitting of the line shape predicts that the high frequency peak does not contain a significant proportion of Lorentzian

component.⁴¹ The temperature dependence of the carboxylate asymmetric stretch band shows that the transition peak not only slightly shifts its maximum to higher frequencies as the temperature increases but also gets slightly narrower at higher temperatures.⁴¹ The modeling of the asymmetric stretch band with temperature reveals that the line shape parameters of the Voigt profiles do not change significantly, and that the spectral variations are caused by a change in the ratio of the band areas.⁴¹ The ratio of the band areas is 0.20 at 20°C , while at 40°C it is 0.13. A similar behavior is observed in the IR spectrum of betaine in ethylene glycol.⁴¹ In this solvent, two transitions are also observed in similar positions to those seen in water; i.e., 1629 cm^{-1} and 1638 cm^{-1} . However, in contrast with water, the two bands have approximately the same area; i.e., the ratio is ~ 0.98 . Moreover, a temperature dependence study (not shown) reveals that the high(low) frequency band decreases(increases) its area when the temperature increases, showing a similar trend as the data reported for water.

To investigate the vibrational components giving rise to the observed line shape, the vibrational spectroscopy of betaine carboxylate asymmetric stretch was further investigated by pump-probe and 2D IR spectroscopy. The vibrational population dynamics of the betaine carboxylate asymmetric stretch was studied by femtosecond pump-probe spectroscopy. The transient spectral response of the betaine carboxylate asymmetric stretch in water after the ultrafast IR excitation is shown in Figure 3. In the signals shown, the neat solvent response was subtracted by measuring the response of the solvent alone. The transient spectrum measured at $T = 0.40\text{ ps}$ (top panel, Figure 3) reveals negative and positive bands that correspond to transitions from $V = 0$ to $V = 1$ vibrational levels and vice versa (i.e., bleach of the ground state and stimulated emission) and from $V = 1$ to $V = 2$ vibrational levels (i.e., excited state absorption (ESA)) of

FIG. 3. Experimental IR transient absorption of betaine. Top panel shows pump-probe spectra of 0.1 M betaine in D_2O at $T = 0.4\text{ ps}$. The lower panel exhibits the transient signal of the photoinduced absorption band (1590 cm^{-1}). Black squares correspond to the experimental data, and the red line to the fitting as is described in the text.

the betaine carboxylate asymmetric stretch. The vibrational anharmonicity (Δ_{12}) of this vibrational mode (i.e., frequency difference between $V = 0$ to $V = 1$ and $V = 1$ to $V = 2$ transitions) was calculated by fitting the transient spectrum at 0.4 ps with two Gaussian functions and it is computed to be approximately 20 cm^{-1} . The temporal dependence of the transient signal at 1590 cm^{-1} shows a dynamics that is not well described by a single exponential decay function, but it is adequately modeled with two exponential decay functions (see Figure 3). The time constants (amplitudes) of the new absorption band are 0.3 ps (0.8) and 1.1 ps (0.4), respectively. The population dynamics observed for betaine shows the same bi-exponential decay behavior presented by a model carboxylate group.¹⁹ Also, the reorientation dynamics (not shown) do not exhibit any appreciable decay in the few picoseconds that were measured accurately. Note that the first 0.1 ps of the signal was not considered due to the presence of other non-resonant signals which cannot be reliably subtracted from the pump-probe signal.

The vibrational dynamics of the betaine carboxylate asymmetric stretch was further characterized by 2D IR spectroscopy. The 2D IR spectra of betaine acquired with pulses having parallel polarizations ($\langle\text{XXXX}\rangle$) at different waiting times, T , are presented in Figure 4. At $T = 0$, the 2D IR spectrum is composed of a set of diagonal peaks, i.e., negative (blue, left) and positive (red, right), which correspond to the different third order non-linear field interactions of the IR pulses with the carboxylate asymmetric stretch of betaine.⁴² Similarly to the pump-probe signal, the negative peak (blue left peak in Figure 4) arises from the pathways involving the excited state absorption (ESA), and the positive peak (red right peak in Figure 4) is due to the bleach/stimulated emission (BSE) pathways. The waiting time evolution of the 2D IR spectra of betaine asymmetric stretch reveals two distinct variations. The first

change is the exponential decay of the peak intensities that is associated with the vibrational lifetime and rotation diffusion.⁴² This effect is not considered in here since it is more accurately characterized by pump-probe spectroscopy. The second variation is the change observed in the peak shapes that are typically associated with spectral diffusion.⁴² At $T = 0$ ps, the positive and negative peaks of the 2D IR spectrum are tilted towards the diagonal ($\omega_\tau = \omega_t$, dashed line in Figure 4) indicating a correlation between the pumped and probed asymmetric stretch frequencies. As the waiting time progresses, $T > 0$ ps, both the negative and positive peaks start to acquire an upright shape from their initial elongated shape along the diagonal showing that the correlation between the pumped and probed asymmetric stretch frequencies vanishes with waiting time. The behavior observed for the 2D IR peaks is a manifestation of the system undergoing strong variations in the environment of the initially excited oscillators (spectral diffusion). Interestingly, the peaks do not acquire a complete upright shape at the longest measured waiting time of $T = 2.5$ ps suggesting that the initially excited asymmetric stretch frequencies cannot be completely randomized by the environment. As is shown below, this is in agreement with the presence of a second transition in the linear IR spectra. In addition, the 2D IR spectra show that the excited state absorption peaks gets narrower as waiting time increases. This change in the excited state band is attributed to the similar magnitude of the betaine photon echo and the water thermal grating signal⁴³ at longer waiting times, which is caused by the short vibrational lifetime of the carboxylate asymmetric stretch population. The lack of band shrinking is corroborated by the similar time evolution of the excited state population at different frequencies from the pump-probe experiments.⁴¹

The function describing the loss of the correlation in the 2D IR spectra is the FFCF.⁴² The frequency–frequency correlation dynamics can be derived from the waiting time

FIG. 4. Absorptive 2D IR spectra of betaine in D_2O . 2D IR spectra for the XXXX pulse polarizations at different waiting times: $T = 0, 0.5, 1.2$, and 2.5 ps. Black dashed line correspond to the diagonal ($\omega_\tau = \omega_t$).

evolution of different spectral components of the 2D IR spectra. Many different methodologies have been derived and used to quantify the FFCF dynamics.^{44,45} Here, the slope of the nodal line (defined as “slope” from here on) between the positive and negative peaks of the 2D IR spectrum at the frequency corresponding to the maximum of the positive peak within the 5 cm^{-1} of the transition is used.⁴⁴ However, similar parameters for the FFCF dynamics are derived when the central line slope⁴⁵ (CLS) approach is utilized.⁴¹ It is important to note that only those FFCF characteristic times (τ_c) fulfilling that $\Delta\tau_c \gg 1$ (where Δ is the amplitude of the fluctuation) for Kubo-like functions⁴⁶ can be extracted from the analysis of the 2D IR spectra.^{44,45}

The waiting time dependence of the slope is presented in Figure 5. As observed, the slope decays in the first picosecond, but it reaches a plateau after $\sim 2\text{ ps}$ which is significantly higher than zero for the longest measured time. The least square fitting of the slope with a function of the form produces the following parameters: $a_1 = 0.21 \pm 0.03$, $\tau_c = 0.42 \pm 0.08$, and $a_2 = 0.06 \pm 0.01$. As metric of the FFCF, the slope reinforces the idea that the initially pumped asymmetric stretch frequencies are not randomized during the measured experimental time. Note that a second experiment shows a similar dynamical behavior of the slope.⁴¹

B. Computations

The gas phase potential energy scan of the dihedral angle, from both the DFT and MP2 electronic structure methods, is shown in Figure 6. The energies from the DFT method and the MP2 are reasonably similar. The potential energy surface is highly symmetric due to the symmetry of the molecule. Hence in calculating free energy profile, US simulations were carried out only from 0° to 90° . The free energy profile for the gas phase betaine at 300 K is shown in Figure 7(a). The results from the condensed phase AIMD studies as well as from the classical force-field simulations are plotted in Figures 7(b) and 7(c), respectively. The free energy profiles obtained using the classical force-field with the smaller and larger system sizes were essentially identical (not shown), indicating that the long range interactions are well represented in the smaller

FIG. 5. Slope versus waiting time for the carboxylate asymmetric stretch of betaine. The black filled squares are the experimental data points and the lines are different modelings. The solid red line corresponds to the fitting with a single exponential decay, and the dotted blue and the dashed magenta lines represent the modeled slope derived from the simulation with two conformers (oscillators) and one conformer (oscillator), respectively.

FIG. 6. The *ab initio* gas phase dihedral (OCCN) potential energy scan at the MP2 level of theory (black line) and the BLYP-D2 level of theory (red line).

system. The condensed phase AIMD simulations show a much smaller barrier of around 1.5 kcal/mol in going from small dihedral angles to 90° , whereas the barrier for the classical force field is around 6 kcal/mol. Interestingly, the gas phase AIMD simulations show a much larger barrier compared to the condensed phase AIMD simulations, indicating the importance of the solvent in lowering of the energy barrier and creating a stable betaine conformation around 90° in the condensed phase.

In order to confirm that there are indeed two populations of betaine species, a dominant one with an OCCN dihedral angle close to 0° (or 180°) and another much smaller one at

FIG. 7. The free energy profile for the OCCN dihedral angles from (a) AIMD simulations of a gas phase molecule, (b) AIMD simulations of the aqueous betaine solution, (c) classical (general amber) force field simulations of the aqueous betaine solution. Error bars were calculated using the block averaging method.

FIG. 8. The distribution of the OCCN dihedral angle from canonical (a) AIMD simulations and (b) classical force field simulations.

90°, the trajectories from the canonical NVT simulations were studied. The distribution of this (unsigned) dihedral angle is shown in Figure 8(a). One clearly sees a small but non-negligible population near 90°, which is completely absent from the force-field simulations (Figure 8(b)).

IV. DISCUSSION

The carboxylate asymmetric stretch of betaine suggests, through the slight asymmetry of the band, the possibility that the peak has two underlying transitions. This idea of the spectrum of betaine in D₂O being composed of two transitions is reinforced by the linear IR spectrum of betaine in ethylene glycol where two transitions are observed, but with different area ratios than in water. In addition, the similar trend in the change of the area ratios with temperature for the two transitions in both solvents makes it unlikely that the assignment of the second transition is either an artifact due to solvent subtraction or a spectral modulation with temperature.

The presence of a second transition can be explained through many different mechanisms. In linear IR spectroscopy, the most common case is related to a Fermi resonance.⁴⁷ A Fermi resonance occurs because a vibrational transition is anharmonically coupled with one overtone or combinational vibrational mode of the same molecule with a similar transition frequency.⁴⁸ Since betaine has 51 vibrational modes, the possibility that betaine has a Fermi resonance is not negligible. However, Fermi resonances are not strongly susceptible to temperature because this will implicate a temperature dependence of the anharmonic coupling between the vibrational mode and the overtone/combinational mode.⁴⁹ The temperature dependence of the asymmetric stretch mode shows that the ratio of the amplitude of the two bands is temperature dependent, i.e., its ratio changes from 0.2 at 20 °C to 0.13 at 40 °C. Thus, this result suggests that the source of the second band is not a Fermi resonance. Moreover, the

asymmetry of the band is also observed for betaine-d11 where it is expected that the vibrational modes and their anharmonic couplings are different.⁴¹ Thus, the asymmetric nature of the band is unlikely to be caused by a Fermi resonance. The second possibility is related to the presence of a second chemical species like an aggregate. Molecular aggregates present different vibrational spectra because the formation of the aggregate significantly alters the solvation environment of the individual molecules. In localized vibrational transitions, the aggregation process usually produces a frequency shift of the vibrational mode as a consequence of the less “polar” environment of the molecule.⁵⁰ However, the formation of molecular aggregates is enhanced by an increase in the temperature because at higher temperatures the entropic part of the hydrogen-bond starts to dominate making the water a poorer hydrogen-bond donor and shifting the equilibrium from monomers to aggregates.⁵¹ This temperature dependence is opposite to what it observed experimentally in the FTIR where the band ratio decreases with temperature. Moreover, there is previous experimental³ and theoretical¹⁰ evidence showing that betaine does not easily form molecular aggregates. The third and last possibility involves the presence of a second conformer of betaine. Since the methyl groups are far from the carboxylate groups, it is unlikely that the conformers related to the torsions involving the tetramethylammonium group will be relevant to the carboxylate vibrational model. On the other hand, the rotation of the carboxylate group about the C–C bond, namely, the OCCN dihedral, is most likely to produce another conformer which could appear in the IR spectrum. The potential of mean force calculated via *ab initio* molecular dynamics simulations confirms the idea that although betaine tends to adopt a conformation in which the dihedral angle OCCN is 0°, a conformer in which the dihedral angle OCCN is 90° also exists in solution. The predicted barrier is ~1.5 kcal/mol which gives rise to a population of around 10% for the 90° conformer. It is important to note that the observables obtained from the *ab initio* molecular dynamics simulation are in good agreement with the linear spectrum of water since the area ratio of the two transitions (0.2) is very similar to the computed ratio of populations (0.1). In addition, DFT calculations of the frequencies of a single betaine in a dielectric medium predict that the frequencies of the 0° and 90° conformers are ~1620 cm⁻¹ and ~1610 cm⁻¹, respectively, for water and ethylene glycol. Although the frequencies appear in a different order with respect to our experimental findings, the small differences in the frequency can be explained either by the error in their determination or by the lack of a real solvent in which processes such as charge transfer could modify significantly the vibrational mode frequency. Moreover, the decrease in the barrier in going from 0° to 90° in the condensed phase AIMD simulations as compared to the gas phase at the same temperature clearly shows that the 90° conformer is stable due to solvent interactions and that reducing the betaine-solvent interactions will lead to the disappearance of this conformer. This theoretical observation explains why the high frequency transition (90° conformation) decreases its area (population) when the temperature is increased in either water or ethylene glycol. As the temperature is increased, water

or ethylene glycol becomes a worse hydrogen bond donor, which not only decreases the betaine-solvent interaction but also shifts the conformational equilibrium of betaine towards the 0° conformer (the most stable conformer).

In a very interesting study, Fornilli and co-workers have carried out QM/MM type simulations of the betaine system in water, using a semi-empirical method, namely, AM1 Specific Reaction Parameters (AM1-SRP) (modified to reproduce the gas phase geometric and energy data of the betaine molecule) for betaine and the TIP3P MM force-field for the water molecules.^{10,12} For comparison, they also used a fully MM model (amber force-field for the water-betaine interactions) that was re-parameterized to reproduce the gas phase *ab initio* torsional energies. The authors did not calculate the free energy profile of the OCCN dihedral angle, but they did plot this dihedral angle as a function of time from a canonical simulation. Their analysis revealed that the betaine OCCN dihedral tended to stay at 0° (or $\pm 180^\circ$) for both the QM/MM as well as MM simulations, though the deviation from 0° (or $\pm 180^\circ$) was larger for the QM/MM method as compared to the full MM simulations. However, their data did not reveal a stable population at 90° . There are two possible reasons for the discrepancy between their QM/MM simulations and the AIMD results presented here. The first is that the *ab initio* treatment in this work is at a different level of theory, namely, DFT, as compared to the semi-empirical treatment in their QM/MM studies. The second reason is the quantum mechanical nature of the water molecules in the AIMD simulations that can play a significant role in the torsional energetics of betaine. Further studies are underway to study the effect of water on the structure and dynamics of betaine as well as the quantum mechanical effects that can give rise to the stabilization of the higher energy OCCN dihedral configurations of betaine in water.

All the experimental and theoretical data point to the presence of a second transition caused by a mixture of betaine conformers. To further demonstrate that this is indeed correct, a model of the vibrational dynamics of the system is presented. A vibrational model requires a complete characterization of all the component of the betaine vibrational dynamics. While the vibrational characteristic times, such as vibrational lifetime and correlation times, are readily available from the experiments, other parameters pertaining to the motionally narrowed components of the FFCF (i.e., not fulfilling $\Delta\tau_c \gg 1$) are not. A list of all the available experimental parameters is presented in Table I. Note that the rate of crossing from one conformer to the other (computed using the high friction limit of Kramer's transition state theory⁵²) is predicted to be very slow; i.e., rate $\sim 1/(250 \text{ ps})$ for the conversion of the 0° conformer to the 90° conformer. Thus, the interconversion of conformers should not significantly affect the modeling of either the linear or the non-linear infrared spectra.

To compute the missing components of the FFCF, a methodology based on the fitting of the FTIR spectrum is used. The idea behind this methodology is that the experimental data contains a lot of redundancy, e.g., the FTIR and 2D IR spectra contain and depend on the FFCF. Note that previously similar methodologies were demonstrated and/or used to obtain the parameters describing the vibrational dynamics

TABLE I. Experimental and theoretical parameters of the vibrational dynamics of betaine. The experimental parameters extracted from experimental results and from the fit of the linear absorption spectra (marked with *).

Line shape parameters	Experimental	Theoretical
$\langle\omega_{10}^1\rangle (\text{cm}^{-1})$	1625.4	...
$\langle\omega_{10}^2\rangle (\text{cm}^{-1})$	1635.0*	...
$\Delta_{12}^1 (\text{cm}^{-1})$	20	...
$\Delta_{12}^2 (\text{cm}^{-1})$	20	...
$\mu_{10}^1 (\text{arbitrary units})$	1	...
$\mu_{10}^2 (\text{arbitrary units})$	1	...
c_1	0.83	...
c_2	0.17	...
$T_{10} (\text{ps})$	0.5 ^a	...
$\Delta_1 (\text{ps}^{-1})$	$1.90 \pm 0.05^*$	1.41 ± 0.05
$\tau_1 (\text{ps})$	0.45	0.27 ± 0.03
$\Delta_2 (\text{ps}^{-1})$	$1.34 \pm 0.05^*$	1.38 ± 0.05
$\tau_2 (\text{ps})$	0.04 ^b	0.010 ± 0.001

^aThis value was computed according to the population relaxation mechanism presented in Ref. 19.

^bValue extracted from the theoretical modeling in Ref. 19.

of the transition (e.g., carboxylate asymmetric stretch of trifluoroacetate,¹⁹ carbon monoxide bind to an enzyme,⁵³ and water⁵⁴), though some of these methodologies rely on both the linear and the non-linear spectra. The linear absorption spectrum $S(\omega)$ of betaine was modeled according to the well-known line shape formalism⁵⁵

$$I(\omega) = 2\text{Re} \int_0^\infty \sum_{j=1}^2 c_j \mu_j^2 \exp(i(\omega - \langle\omega_{10}^j\rangle)t) \times \exp(-g(t) - t/2T_{10} - 2Dt) dt, \quad (1)$$

where c_j is the concentration of the j -th conformer, μ_j is the transition dipole magnitude of the j -th conformer for the $v = 0$ to $v = 1$ transition, $\langle\omega_{10}^j\rangle$ is the transition frequency of the j -th conformer, $g(t)$ is the double time integral of the FFCF (i.e., $g(t) = \int_0^t d\tau_1 \int_0^{\tau_1} \langle\delta\omega_{10}(\tau_2) \delta\omega_{10}(0)\rangle d\tau_2$), T_{10} is the vibrational population relaxation time, and D is the rotational diffusion coefficient. The concentrations (c_i) used, 0.83 and 0.17, are those retrieved from the areas of the FTIR fit with two Voigt profiles (see Sec. II). The transition dipole magnitudes are assumed to be the same. This assumption has been validated by DFT calculations in which the 0° and 90° conformations are predicted to have transition dipole magnitudes, for the carboxylate asymmetric stretch, of 551.4 km/mol and 509.6 km/mol, respectively. Although the central frequencies can be retrieved from the FTIR fit, a better modeling of the FTIR is obtained when the central frequency corresponding to the conformer having the 0.17 concentration is used as a fitting parameter. The vibrational population relaxation time was computed from the proposed population kinetic mechanism in Ref. 19 which gave a T_{10} of 0.5 ps. The rotational diffusion coefficient, D , is set to zero because there was no measurable reorientation dynamics in the experiments. In the case of the FFCF, it is assumed here that both conformers have the same functional form, since there is not enough experimental and/or theoretical evidence to make them different. As presented in Figure 5, the experimental data

suggest a FFCF model correlation function of the form

$$\langle \delta\omega_{10}(t) \delta\omega_{10}(0) \rangle = \Delta_1^2 \exp(-t/\tau_1) + \Delta_2^2, \quad (2)$$

which contains one characteristic time (i.e., τ_1) that is assigned to the hydration dynamics associated with the translation motions of water and the static inhomogeneous term (Δ_2). Although it is possible that the FFCF contains a static inhomogeneous term; in this case, the static inhomogeneous term is a consequence of the presence of a second transition (see below). The effect of a second transition in the dynamics of the slope has been previously observed in different studies where it has been shown that a second transition creates a static inhomogeneous component in this metric.^{19,56} The physical interpretation of the effect of a second transition is as follows: a second transition, even if it has the same correlation times as the other transition, produces a static inhomogeneous component because the population of the two transitions will not interchange during the experimentally measured time. Moreover, previous theoretical modeling of the carboxylate asymmetric stretch predicted that the FFCF does not contain a static inhomogeneous component and has a functional form

given by¹⁹

$$\langle \delta\omega_{10}(t) \delta\omega_{10}(0) \rangle = \Delta_1^2 \exp(-t/\tau_1) + \Delta_2^2 \exp(-t/\tau_2). \quad (3)$$

In this expression of the FFCF, the first and second exponential terms have been assigned to the translation and libration motions of water, respectively.¹⁹ Since the amplitude and characteristic time associated with the libration motion of water do not fulfill that $\Delta\tau_C \gg 1$, both parameters must be included in the fitting. However, there is enough experimental and theoretical modeling suggesting that the characteristic time of the libration motion is approximately 50 fs.²¹ Thus, a characteristic time of 40 fs is used to describe the libration dynamics since it has been predicted theoretically in the hydration dynamics of a model carboxylate.¹⁹

The results of the FTIR fitting are presented in Figure 9. Even though only three parameters (i.e., $\langle \omega_{10}^2 \rangle$, Δ_1^2 , and Δ_2^2) were used to fit the experimental linear absorption spectrum, the fitted FTIR spectrum shows an excellent agreement with the experiment. To further validate the fitting methodology used in here, the same set of parameters is used to simulate the 2D IR spectra and their waiting time evolution. The model used here to simulate the 2D IR spectra has been described in detail elsewhere.⁵⁷ As depicted in Figure 9, the

FIG. 9. FTIR and absorptive 2D IR spectra of betaine in D₂O. The top panels correspond to the experimental (black squares), simulated FTIR spectra (red line), and residuals of the model using one and two transitions as described in the text. The bottom panels show the experimental (left column) and simulated (center and right columns) 2D IR spectra at different waiting times: T = 0 (top panels) and 1.2 ps (bottom panels). The black lines show the zeroth contour line (slope) and the CLS.

simulated 2D IR spectra are in very good agreement with the experiment.⁴¹ Note that the peak corresponding to the new absorption signal does not match with the experimental result very well. This mismatch is most likely a result of using the rate of the population relaxation as twice that of the ground state.⁵⁷ This assumption might not be valid for a carboxylate where it is known that the carboxylate symmetric stretch transition participates in the vibrational relaxation of the first excited state.¹⁹ Interestingly, the simulated data also show that the slope and the CLS are not straight lines, i.e., they have curvature. The curvature of these two metrics arises from the presence of a second transition. This last statement is verified by following the same analysis procedure used to retrieve those missing parameters of the line shape function, but including only one transition in the modeling of the FTIR and 2D IR spectra. The results (Figure 9) show that when the system is represented by a single transition, no curvature is observed either in the slope or in the CLS even if a static inhomogeneous component is purposely added to the FFCF.⁴¹ Moreover, it is apparent that the CLS is more susceptible to the presence of a second transition than the slope. In addition, the peaks of the simulated 2D IR spectra with one transition has a more Lorentzian shape as seen by the elongated (triangular) shape of the red peak along the ω_r axis especially at longer waiting times. Thus, the analysis of the features of the 2D IR spectra allows us to assess that the vibrational band corresponding to the carboxylate asymmetric stretch of betaine is indeed composed of two transitions.

In addition, the slope from the simulated spectra was calculated (Figure 5 bottom panel). The simulated slope also exhibits a good agreement with the experimental data. It is important to note that the betaine FFCF (see Eq. (3) and Table I) does not include a static inhomogeneous component. Thus, the presence of a long time component in the slope is a consequence of the presence of a second transition. Moreover, as in the simulation of the 2D IR plot, a model that includes only a single transition does not reproduce as well the experimental slope even when the FFCF contains a static inhomogeneous component (see Figure 5 and Ref. 41). In addition, the dynamics of the simulated slope also shows a decay of 0.44 ps demonstrating that in this case the presence of a second transition does not significantly perturb the dynamics of this metric.

From the simulation of the 2D IR spectrum, it is now clear that the characteristic time of 0.45 ps measured from the slope truly represents the hydration dynamics of the system due to reorientation motions of the water molecules not including librations. Surprisingly, this correlation time is faster than observed for the model carboxylate.¹⁹ Moreover, *a priori* one would have expected that the comparative closeness of the methyl groups of the tetramethylammonium group and the charged carboxylate group would have slowed the hydration dynamics of the whole molecule as has been predicted in previous theoretical work.¹¹ To verify our interpretation of the dynamics of the slope describing the hydration dynamics, the FFCF is computed from the *ab initio* molecular dynamics trajectories using the DFT frequency map (see Sec. II). As presented in Figure 10, the computed FFCF from molecular dynamics simulations shows two different dynamics occurring

FIG. 10. Autocorrelation of the frequency fluctuations calculated from the AIMD/DFT map. The solid black line is the FFCF and the dashed red line is the fit with two exponential functions. Inset shows the first 500 fs of the autocorrelation of the frequency fluctuations.

in the first 2.5 ps and no slow correlation decay and is in agreement with the FFCF parameters retrieved from the experiments. The value of the characteristic times (τ_c) and amplitudes of the fluctuations (Δ_i) calculated by modeling the dynamics of the simulated FFCF with two exponential decay functions are presented in Table I. The first component has a characteristic time of 0.01 ps and is motionally narrowed, and the other component has a characteristic time of 0.27 ps but arises from a dynamic inhomogeneous distribution of frequencies associated with the reorganization of the solvent shell. These values of the amplitudes of the frequency fluctuations as well as the characteristic times predicted by the theory are in good agreement with those retrieved from the analysis of the experimental data (Table I). Moreover, it has been previously observed that the correlation times predicted by DFT maps are faster than those seen experimentally¹⁹ which might explain the differences seen here. Although the computation of the FFCF with a map does not include fluctuations of the frequency due to changes in the betaine dihedral angle, DFT calculations predict that the changes in the vibrational frequency with the OCCN dihedral angle of betaine are very small (~ 10 cm⁻¹ from 0° to 90°). Thus, it is fair to assume that the changes in the frequency due to variations in the betaine geometry will not have a significant effect on the FFCF.

Betaine has a unique hydration dynamics since the characteristic time of the water reorganization motions is the fastest measured for a single and uncoupled vibrational transition in water (see, for example, Refs. 19 and 58–63). There are many different mechanisms that can explain the characteristic times observed in the FFCF. One possibility is that the characteristic times of betaine hydration dynamics are related to the fact that betaine does not have a counter ion that could alter the solvation dynamics, like in the case of the other measured carboxylates.^{17,19} Another possibility is related to the charged structure of betaine. Betaine has a very strong dipole because of its zwitterionic nature.⁷ Therefore, it is conceivable that the small changes in the conformation of betaine, especially those that slightly change the distance between the carboxylate and the ammonium groups, modify the dipole and consequently the orientation of the water molecules in the solvation shell. This dipole

effect might be similar to what is produced by an electronic excitation of a molecule where the sudden change in the dipole direction produces the reorganization of the solvation shell.⁶⁴ Thus, fast fluctuations around the equilibrium positions of OCCN dihedral angle (i.e., 0° and 90°) are likely to induce changes in the solvation shell. The last possibility involves quantum mechanical effects such as charge transfer. In this case, the association of the water molecules with betaine could be perturbing the electronic structure of the whole molecule and consequently how the molecule associates with its solvation shell. Thus, the formation of a hydrogen-bond could induce a change in the charge molecular distribution⁶⁵ which will directly affect the hydration shell. Our AIMD simulations predict a hydrogen bond lifetime of ~3 ps (using the O(carboxylate)-H(water) distance as the parameter for the definition)⁶⁶ for those water molecules in the solvation shell of the carboxylate group. This result is not inconsistent with the observed hydration dynamics since the fluctuations of the frequency can arise from reorganization motions in the hydration shell that do not necessarily require a change in the number of hydrogen-bonded water molecules as predicted for another carboxylate ion.¹⁹ Moreover, it has been shown that a slight change in the water orientation with respect to the carbonyl group has a significant effect on the charge transfer.⁶⁷ Since these different mechanisms can give rise to the vibrational dynamics observed here, an analysis of the complex collective motions of water and its effect on the molecular characteristics of the betaine zwitterion are beyond the scope of the present work and will be dealt with in a future publication.

V. CONCLUSION

The vibrational spectroscopy of the carboxylate asymmetric stretch of aqueous betaine was fully characterized. The band located at ~1625 cm⁻¹ is assigned to this transition and has an asymmetric shape due to the presence of a second transition. The presence of two types of conformers, a dominant one with an OCCN dihedral of 0° and a second at 90° with a smaller population, from AIMD simulations suggests that the second transition is caused by the presence of the second betaine conformer.

Betaine also shows similar population dynamics as observed in other carboxylates. A fitting model, involving the FTIR and parameters derived from linear and non-linear spectroscopies, was successfully used to retrieve missing parameters of the FFCF, such as those related to motional narrowing. The modeling of non-linear spectra with the derived FFCF parameters demonstrates that the shape of 2D IR spectra and its evolution are consistent with the band being composed of two transitions. In addition, the modeling shows that 2D IR spectral parameters, such as the slope or the CLS, are sensitive to the presence of a second transition. In particular, the presence of a second transition is readily observed through the curvature of the CLS in the 2D IR.

Our modeling of the experimental data also shows that the solvent induced frequency fluctuations exhibited by betaine are on ultrafast time scales (~0.45 ps) and are one of the fastest correlation times measured for single localized transition in

water. This ultrafast decay of the FFCF characteristic time was corroborated by computations of the FFCF from AIMD trajectories using a DFT map, which in turn gave a value of ~0.3 ps for the ultrafast relaxation time.

ACKNOWLEDGMENTS

One of the authors (D.G.K.) would like to acknowledge Professor Robin M. Hochstrasser for his helpful discussions that led to this project. For R.K., the current work is funded in part by the NSF EPSCoR LA-SiGMA project under Award No. EPS-1003897, and a Louisiana Board of Regents (BOR RCS Grant No. LEQSF(2014-17)-RD-A-02) and startup funds provided by the Department of Chemistry and the Center of Computation Technology at LSU. For D.G.K., the current work is funded in part by the NSF EPSCoR under Award No. EPS-1003897 Louisiana Board of Regents (BOR RCS Grant No. LEQSF-EPS(2015)-PFUND-396), and startup funds provided by the Department of Chemistry and by Grant No. NIH-9P41GM104605. All the authors would like to acknowledge the High Performance Computing Center at LSU for computer time.

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