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Effect of Osmolytes on the Conformational Behavior of a Macromolecule in a Cytoplasm-like Crowded Environment: A Femtosecond Mid-IR Pump−Probe Spectroscopy Study

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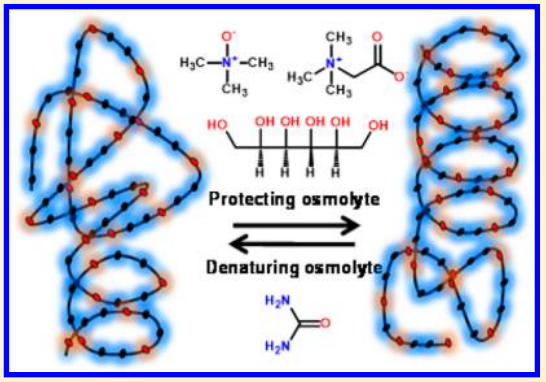
Supporting Information

ABSTRACT: Osmolytes found endogenously in almost all living beings play an important role in regulating cell volume under harsh environment. Here, to address the longstanding questions about the underlying mechanism of osmolyte effects, we use femtosecond mid-IR pump−probe spectroscopy with two different IR probes that are the OD stretching mode of HDO and the azido stretching mode of azido-derivatized poly(ethylene glycol) dimethyl ether (PEGDME). Our experimental results show that protecting osmolytes bind strongly with water molecules and dehydrate polymer surface, which results in promoting intramolecular interactions of the polymer. By contrast, urea behaves like water molecules without significantly disrupting water H-bonding network and favors extended and random-coil segments of the polymer chain by directly participating in solvation of the polymer. Our findings highlight the importance of direct interaction between urea and macromolecule, while protecting osmolytes indirectly affect the macromolecule through enhancing the water−osmolyte interaction in a crowded environment, which is the case that is often encountered in real biological systems.



Almost all living organisms need to adapt to environmental stresses such as extreme pressure, temperature, pH, cellular dehydration, high extracellular salt, and the presence of denaturing highly concentrated urea and salts inside cells like those in the mammalian kidney.[1,2] Cells adapt to such stresses by accumulating certain small organic molecules, known as osmolytes. Osmolytes are known to have profound effects on protein stability. A few of them are denaturants like urea, whereas others like trimethylglycine (TMG), trimethylamine N-oxide (TMAO), etc. act as protecting osmolytes even under denaturing conditions by stabilizing native protein structures. The ability of TMAO to promote protein folding was interestingly used to study the mechanisms of mis-folding processes of disease-causing proteins like prion protein,[3] tau protein (related to Alzheimer's disease),[4] and α-synuclein.[5,6] An important question that has not yet been fully elucidated is how osmolyte molecules affect protein stability in living cells? A number of studies emphasized the importance of either *direct* interactions between osmolyte molecules and protein via hydrogen bonds (H-bonds) and other electrostatic interactions or favorable van der Waals interactions between osmolyte molecules and protein's amino acids, which essentially shift the equilibrium between folded and unfolded protein structure toward its folded (native) state.[7−12] Other studies, however, showed that osmolyte molecules modulate or disrupt the H-bonding network of water, which then indirectly induces changes in the delicate balance between the forces and entropies of folded and unfolded protein states. As a result, the stability of the protein is modified.[13−18]

In the case of urea, recent time-resolved IR and vibrational sum-frequency-generation spectroscopic studies showed that urea does not have any long-range effect on the fluctuating H-bonding network and structural dynamics of water.[8,19,20] Only a small fraction of water molecules that are doubly H-bonded to urea exhibit slow reorientation dynamics compared to those in bulk water. By contrast, TMAO, an important protecting osmolyte, interacts with water molecules strongly and has a tendency to slow down the rotation of water molecules close to its hydrophobic part.[17,21−23] However, all the previous time-resolved vibrational spectroscopic studies aimed at revealing osmolyte effects considered binary aqueous solutions consisting of water and osmolyte molecules without any other cosolute molecules. In a real biological system, osmolyte molecules are dissolved in complicated and crowded solution, e.g., intracellular environment, which contains various finite-sized molecules constituting 30−40% of the total cell mass.[24]

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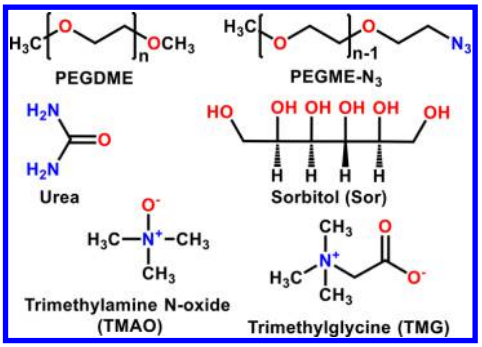
Understanding the mechanism of osmolyte effects in such a crowded environment instead of simple osmolyte−water solutions is thus of significance in elucidating their biophysical roles. Nonetheless, since it is almost impossible to carry out time-resolved IR studies on osmolyte effects *in vivo*, we here consider aqueous osmolyte solutions with highly concentrated macromolecules. Using two different IR probes, namely, deuterated water and azido-derivatized polymer, in combination with thr femtosecond mid-IR pump−probe (PP) method, we address two important questions: (i) what are osmolyte effects on fluctuating water H-bonding network structure as well as on vibrational population and rotational dynamics of water? and (ii) do osmolyte molecules affect the conformation of macromolecule by directly interacting with the polymer or indirectly changing water H-bonding network?

Here, the polymer of interest is poly(ethylene glycol) dimethyl ether (PEGDME: Scheme 1) composed of a repeating unit of ethylene oxide ($-O-CH_2-CH_2-$). PEG is a well-known macromolecular crowder due to its high water solubility and its ability to modify the nature of the interaction between its ether oxygen and water by altering its local dipole moment through dihedral rotations along the C−C and C−O bonds of each ethylene oxide unit. In fact, PEG chain segments can adopt a number of conformations due to the rotations along those single bonds in each $O-CH_2-CH_2-O$ unit.[25,26] The previous studies have demonstrated that the preferred conformation in the $O-CH_2-CH_2-O$ segment is trans−gauche−trans (*tgt*) form among various possible conformations with marginally different energies. The *tgt* conformation for the $O-CH_2-CH_2-O$ segment introduces a helical structure formation in the PEG chains. The preference of the gauche conformation around the central C−C bond, i.e., *tgt* conformation (in $O-CH_2-CH_2-O$ segment), is mainly attributed to three possible factors. The first is H-bonding between the ether oxygen atoms of the PEG chain and water, where a single water molecule bridges between the adjacent ether oxygen atoms separated by one oxyethylene (OE) unit. In addition, one or two water molecules can act as a bridge connecting between two ether oxygen atoms separated by two OE units. The second factor is related to the observation that the distance between the oxygen atoms in the $O-CH_2-CH_2-O$ segment in the gauche conformation is approximately 0.29 nm. This coincides with the nearest-neighbor oxygen−oxygen distance, 0.285 nm, in liquid water.[27] Therefore, the PEG unit segments do not strongly perturb water H-bonding network structure. The third is an electrostatic factor. Because the $O-CH_2-CH_2-O$ segment in the gauche conformation has a larger dipole moment than the same segment in the trans conformation, it hence interacts more favorably with water. In addition, *tgt* conformer allows all the ether oxygen lone pairs into the center of a helical structure, without overlapping, which allows more efficient packing of the segments as the intermolecular repulsion is minimized. Any change of dihedral conformations, such as C−C bonds with a trans conformation and C−O bonds with a gauche confirmation, causes the helical structure to become random-coil-like structures so that both helical and random-coil-like conformers could coexist in solution. This conformational change among the segments due to PEG's ability to alter its local dipole moment through dihedral rotations along the C−C and C−O bonds modifies its interaction with surrounding molecules.

Scheme 1. Molecular Structures of Poly(ethylene glycol) Dimethyl Ether (PEGDME) and Its Azido Derivative ($PEGME-N_3$)[a]



[a]The chemical structures of urea, trimethylamine *N*-oxide (TMAO), trimethylglycine (TMG), and sorbitol (Sor).

Recently, we have shown that the femtosecond IR PP measurement method is of use to identify two distinctively different water molecules: those interacting with other water molecules (OD−w) and the others interacting with ether oxygen atoms of PEGDME (OD−e).[25] In the case of PEGDME, it is known that, in water−rich solutions, they adopt extended conformations with a large population of random−coil−like segments, while, in water−deficient solutions, they favor helical segments because ether oxygen atoms need to adopt to a reduced number of H-number partners (water molecules). Our IR PP studies of the OD stretching mode of 5% HDO in highly concentrated PEGDME solutions showed that the vibrational properties and populations of these two water species is in correlation with conformational transition of PEGDME from random-coil to compact and approximately helical structure above 50 wt % concentration of PEGDME in water.[25]

In the present work, the main goal of research is to find experimental evidence for elucidating the underlying mechanism of both denaturing and protecting osmolyte actions in macromolecular crowded environments, where two different IR-probes, namely, the OD stretching mode of HDO and the azido stretching mode of an azido-derivatized PEGDME ($PEGME-N_3$: Scheme 1), are used. The OD stretching mode of HDO in isotopically diluted water (2.5% D_2O in H_2O) is an ideal IR probe for monitoring highly local structural dynamics of water.[19,25,28−32] The azido stretching mode of $PEGME-N_3$ with exceptionally large IR dipole strength provides information on the local hydration environment around the azido-derivatized PEG, which could vary from one osmolyte to the other.[19,25,33−35] The concentration of PEGDME in all aqueous solutions is 50 wt %, which mimics the crowded environment in cytoplasm of living cells. Note also that it is this 50 wt % concentration of PEGDME that is close to the critical concentration associated with its conformational transition from random-coil to helical structures. Three different types of protecting osmolytes considered in the present study are amino oxide (TMAO), amino acid derivative (TMG), and polyol (sorbitol), while the denaturant is urea (Scheme 1).

IR Absorption Spectra. In binary water−osmolyte solutions, osmolyte molecules except urea cause a red-shift of the OD stretch IR spectrum (Figure S1), which results from redistribution of equilibrium H-bond numbers and H-bonding strengths.[28,36,37] The FTIR spectra of the OD stretching mode

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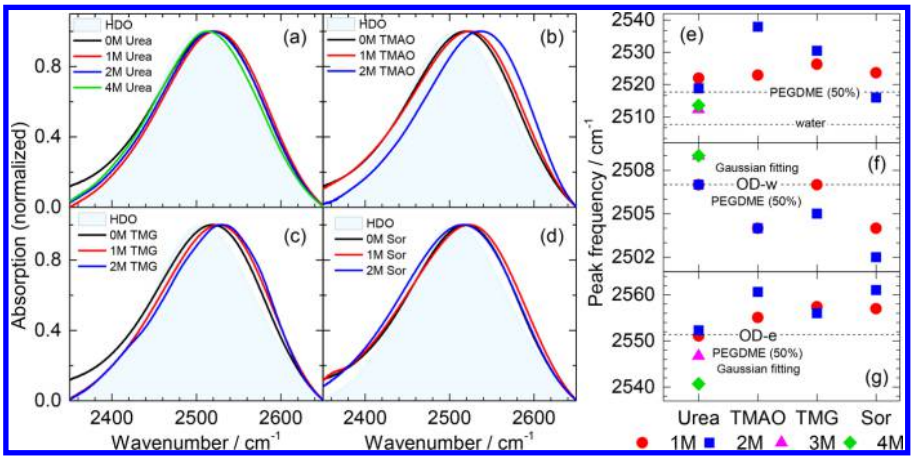

Figure 1. FTIR spectra of the OD stretching mode of HDO in 50 wt % PEGDME solution (blue shaded area). The OD stretch IR spectra of HDO in 50 wt % PEGDME solutions with urea (a), TMAO (b), TMG (c), and sorbitol (d). The peak frequencies for different osmolytes are plotted in panel e. The horizontal dash lines in panel e correspond to the peak frequencies of the OD stretching mode in neat water (lower dashed line) and aqueous 50 wt % PEGDME solution without osmolytes (upper). FTIR spectrum at each osmolyte concentration was fitted with a Gaussian function with two different peaks that correspond to OD−water (OD−w, fixed at values taken from the binary solutions of osmolyte-water) and OD−ether (OD−e), respectively (see Figure S2 for fitting results). The dashed lines in panels f and e are the peak frequencies of the OD−w and OD−e species in aqueous 50 wt % PEGDME solution, respectively, which were obtained by carrying out fitting to the FTIR spectrum with two Gaussian functions.

in ternary water−osmolyte−PEGDME (50 wt %) solutions are presented in Figure 1. The peak position of the OD stretch IR band is independent of added urea concentration in 50 wt % PEGDME solution (Figure 1e). By contrast, as the concentration of protecting osmolytes increases, the OD stretch band of 5% HDO + 50 wt % PEGDME solution undergoes a blue-shift except sorbitol (Figure 1e). In aqueous solutions of PEGDME, HDO molecules interact with either other water molecules (OD−w) or ether oxygen atoms of PEGDMEs (OD−e).

To measure the frequency shifts of the OD stretching modes of OD−w and OD−e water species separately, each OD stretch IR spectra was fitted with two Gaussian functions (Figure S2). Our recent work on PEGDME has shown that the peak position of the OD−w isotropic PP spectra is independent of PEGDME concentration.[25] Therefore, the Gaussian peak position of the OD stretch band of OD−w species at a given osmolyte concentration in 50 wt % PEGDGE solution was set to the same value obtained from the corresponding binary osmolyte−water solution without PEDGME[19] at that particular osmolyte concentration. An important observation here is that the peak shifts of both OD−w and OD−e components are anticorrelated with each other. For example, urea causes the OD−e peak position to shift to low frequency, while protecting osmolytes (TMAO, TMG, and Sor) make the OD−e peak position shift to high frequency (Figure 1f). Such anticorrelation in OD−e stretch frequency shifts suggests that the surface of PEGDME is well hydrated in urea solution, whereas protecting osmolytes dehydrate surface region of PEGDME, similar to what happens in high concentrations of PEGDME.[25]

Another IR probe considered here is the azido stretch mode of PEGME−N_3. The peak frequencies of the azido stretching mode of PEGME−N_3 in 50 wt % PEGDME for varying osmolytes provide information on local structure and effective concentration of water molecules. Figure 2 presents the azido stretch IR spectra of PEGME−N_3 in 50 wt % PEGDME + osmolyte solutions. It has been known that the azido stretching mode frequency does not strongly depend on solvent polarity but does depend on the number and orientation of H-bonded water molecules; however, the number density of H-bonding

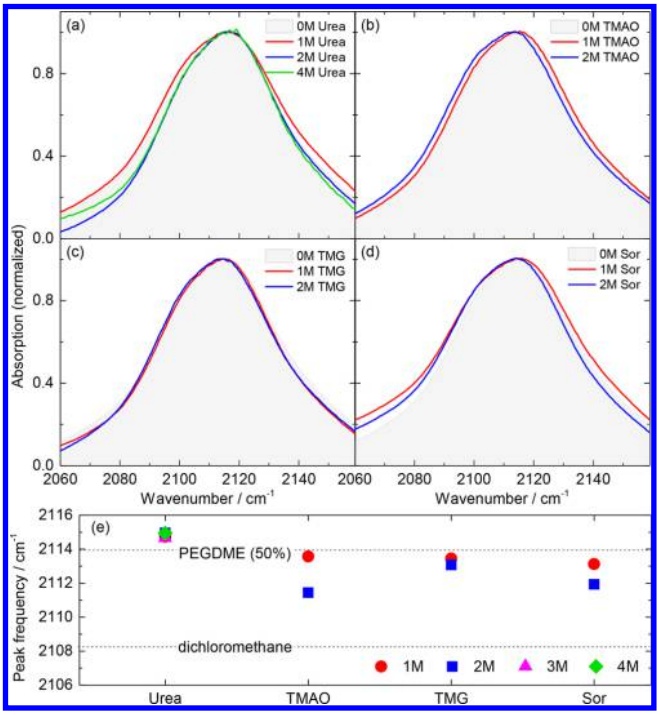

Figure 2. FTIR spectra of the azido stretching mode (of PEGME−N_3) in aqueous 50 wt % PEGDME (gray shaded area) solution without osmolytes. The azido stretch IR spectra of PEGME−N_3 in aqueous 50 wt % PEGDME solutions with added urea (a), TMAO (b), TMG (c), and sorbitol (d). The peak frequencies are plotted in panel e. The peak frequency of the azido stretching mode of PEGME−N_3 is obtained with pseudo-Voigt function fitting (see Figure S3 for details on fitting). The horizontal dash lines correspond to the peak frequencies of the azido stretching mode in dichloromethane (lower line) and 50 wt % PEGDME (upper line), respectively.

partners (water molecules) appears to be the dominant factor in determining the solvatochromic azido frequency shift.[33] Bearing this aspect in mind, let us examine the azido IR stretch bands more in detail. First of all, it is observed that the peak frequency of the azido IR band of PEGME−N_3 appears to be

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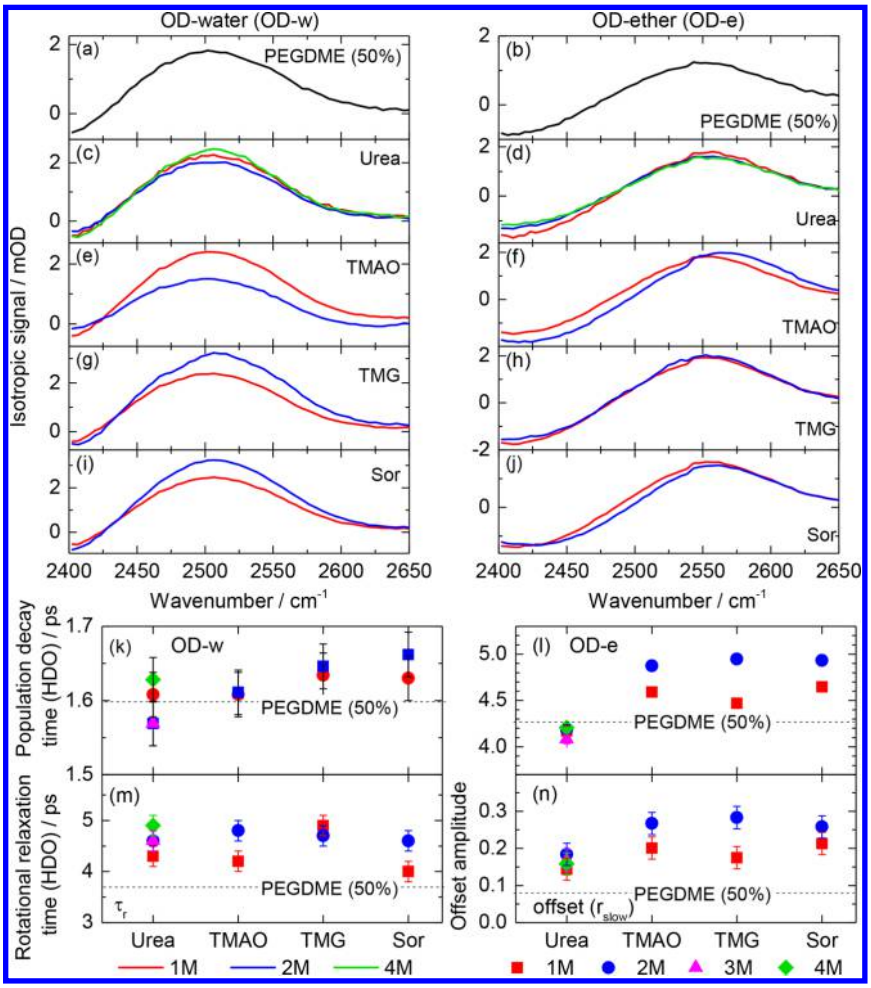

Figure 3. Vibrational population relaxation of the OD stretching mode is biexponential in aqueous 50 wt % PEGDME solutions, and the fast and slow components can be assigned to those of OD−w and OD−e, respectively. The decomposed isotropic PP spectra of the OD stretching mode in aqueous 50 wt % PEGDME solutions with osmolytes are shown in panels a−j. The measured vibrational lifetimes and rotational relaxation times of the OD stretching mode are plotted in the lower panels (k−n). The vibrational lifetimes of the OD stretching mode of OD−w species neither depends on osmolyte type nor its concentration. Details of the heating correction procedure can be found in the SI (Figure S6). The anisotropy decays at the center frequency of the OD stretching mode were fitted to a single exponential function (with time constant of τ_r) with an offset (r_{slow}), i.e., $r(t) = r_0 \exp(-t/\tau_r) + r_{slow}$ (Figure S8). Here, r_0 and r_{slow} are approximately the populations of OD−w and OD−e species, respectively. The error bars signify error range obtained while fitting the raw data.

negligibly dependent on urea concentration (Figures 2a,e), which indicates that the surface region of each PEGDME remains well hydrated even in the presence of urea. This further shows that urea molecules that are known to be compatible with water molecules can even form H-bonding interactions with PEGDME. By contrast, the addition of protecting osmolytes to 50 wt % PEGDME (with PEGME−N_3) induces a red-shift of the azido stretch IR band. This is strong evidence that the surface of PEGDME is dehydrated, compared to the hydration state of PEGDME in 50 wt % PEGDME solution, by the dissolved osmolyte molecules. The extent of dehydration near macromolecule's surface region is largest in TMAO and smallest in TMG. This is in excellent agreement with previous studies, where both TMAO[21] and sorbitol[19] have long-range effects on water H-bonding network and dynamics while TMG[19] perturbs water H-bonding network only in its vicinity.

Vibrational Population Relaxation. To further confirm the existence of two distinctively different groups of water molecules, i.e., OD−w and OD−e, the vibrational relaxation analyses of IR PP data were performed. Here, it should be noted that the vibrational lifetime is determined by the distribution and strength of coupled intra- and intermolecular modes.[38] In the 50 wt % PEGDME solution with 5% HDO, the vibrational lifetimes of the two water species, OD−w and OD−e, are expected to be different. The isotropic IR PP spectrum (see Figure S4 for two representative isotropic IR PP spectra) of the OD stretch mode exhibits both positive peak at 2520 cm^{-1} originating from ground-state bleach (GSB) and stimulated emission (SE) contributions and negative peak below 2430 cm^{-1}, which is associated with excited−state absorption (ESA) contribution. The overall decay of the isotropic IR PP signal can be fitted with two exponentially decaying components and one ingrowing heating component (see Supporting Information for fitting results). The decomposed isotropic IR PP eigenspectra of the bulk-like water component (OD−w) and the water interacting with PEGDME (OD−e) in various osmolyte solutions are shown in Figure 3.

Water's Point of View. One of the most important observations in the present work is that the decay times of the fast component (OD−w) in 50 wt % PEGDME solution are almost independent of the concentration and type of osmolytes (Figures 3k and S5). This is in fact consistent with our previous

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work in the binary osmolyte−water solutions without macromolecular crowders.[19] Although the water H-bonding network is perturbed by the presence of osmolyte molecules (FTIR; Figure 1) in 50 wt % PEGDME solution, a significant population of water relaxes on a time scale similar to that in pure water. This strongly indicates that the strong and robust water−water H-bonds are present even in such a macromolecule-crowded aqueous solution, in addition to those water molecules interacting with ether oxygen atoms of PEGDMEs.

Unlike the fast lifetime component (OD−w), the decay time of the slow component (OD−e) becomes increasingly slower with increasing concentration of protecting osmolytes dissolved in 50 wt % PEGDME solution (see Figure 3l). We believe that the dependence of OD−e lifetime on the nature of osmolyte is important in understanding the role of osmolyte molecules on the hydration of macromolecule. Interestingly, the vibrational lifetimes of both the OD−w and OD−e components are independent of the urea concentration (Figure 3k,l). This again supports the notation that urea and water are interchangeable, and furthermore urea molecules are equally effective in solvating ether oxygen atoms of the macromolecule. By stark contrast, protecting osmolytes significantly slow down the vibrational relaxation of OD−e, which can be evidence that they dehydrate the surface region of PEGDME. To explain this, let us consider the number of water molecules per ether group in 50 wt % PEGDME solution (with no osmolyte), which is estimated to be approximately 2. The average number of water molecules per ether group decreases to 1 at 70 wt % PEGDME. Experimentally, we found that an increase in wt % of PEGDME slows down the vibrational population relaxation of the OD−e species. More specifically, the vibrational lifetime of the OD−e component increases from 4.3 ± 0.2 to 5.1 ± 0.2 ps on increasing concentration of PEGDME from 50 to 70 wt %.[25] In the present work with a fixed PEGDME concentration, we observed that adding protecting osmolytes to the (50 wt %) PEGDME solution also slows down the vibrational lifetime of the OD−e component from 4.3 ± 0.2 ps (0 M osmolyte; 50 wt % PEGDME) to 4.9 (2 M TMAO) or 5.0 (2 M TMG) or 4.9 (2 M Sorbitol) ps. Therefore, the added osmolyte molecules, similar to the case that the concentration of PEGDME is increased, induce a significant dehydration of the surface of PEGDME. Such osmolyte-induced dehydration of the macromolecule would result in a greater cooperation and promotion of intramolecular interactions between the CH_2 segments and the ether oxygen atoms as well as water-bridging H-bonding interactions between the ether oxygen atoms of the PEG chain. In addition, dehydration of PEG surface water will enhance H-bonding interactions between inner water molecules within the helical segments in the macromolecule. All these factors combined induces conformational changes of PEGDME upon increasing osmolyte concentration.

For PEGDME, the structural change could be related to an increasing heterogeneity in the conformations of O−CH_2−CH_2−O segments or a change in equilibrium between the populations of helical and random-coil segments.[39,40] Such conformational shift toward helical structures upon adding protecting osmolytes to the PEGDME solution is similar to the case in water-deficient solution, where the PEG macromolecules tend to adopt crystalline structures similar to solid PEG.[40] As shown below, direct evidence of considerable change in the conformation of PEGDME as protecting osmolyte concentration increases can be inferred from the mid-IR PP measurements of IR-probe-labeled macromolecule itself (PEGME−N_3).

Water Orientational Relaxation. Polarization-selective mid-IR PP measurements provide critical information on rotational times of water (HDO) molecules (see SI for experimental details). For HDO in neat water, it was shown that the anisotropy $r(t)$ decays exponentially with a time constant of 2.3 ± 0.2 ps.[28] The orientational relaxation of each individual water molecule involves both breaking and reforming of H-bonds of the water molecule in a bifurcated H-bond configuration with two other water molecules (one incoming and the other outgoing).[41] Any hindrance to such processes either by reduced number of H-bonding partners or excluded volume due to neighboring hydrophobic molecules can contribute to the slowdown of water rotation.[36,42] In the 50 wt % PEGDME solution, the orientational relaxations of both the OD−w and OD−e species are affected by the presence of PEGDME. Here, the anisotropy decay ($r(t)$) is fitted with one exponential function and a constant offset, i.e., $r(t) = r_0 \exp(-t/\tau_r) + r_{slow}$, where τ_r and r_{slow} (offset) represent the rotational time constant of OD−w and the anisotropy amplitude of slowly rotating OD−e (>10 ps), respectively. The constant offset signifies the population of water molecules that are H-bonded to ether oxygen atoms of the macromolecule−note that the rotational time of the OD-e species cannot be measured within the experimental time window determined by the vibrational lifetime of the OD stretching mode of OD−e. The rotational time constant τ_r of OD−w increases marginally with increasing concentration of urea (up to 4 M) and the offset remains almost independent of urea concentration (up to 4 M). This observation again supports our finding that PEGDME in aqueous urea solutions remains well solvated (hydrated) by sufficient amount of water molecules even in the presence of dissolved urea molecules.

In the cases of the protecting osmolytes, the offset value is not only concentration-dependent but also much higher than that in the 50 wt % PEGDME solution without protecting osmolyte molecules. The higher offset values mean that protecting osmolytes dehydrate the surface region of PEGDME, which is quite similar to those water-deficient solutions in a highly crowded aqueous environment. The reason for the increase in the value of the slow component (offset) is mostly due to strong osmolyte−water H-bonding interactions, which then reduces the probability of breaking and reforming of H-bonds of water molecules in the surface region of the macromolecule.

Macromolecule's Point of View. The isotropic IR PP spectra of PEGME−N_3 consists of a positive peak originating from GSB and SE at around 2120 cm^{-1} and a negative ESA peak below 2070 cm^{-1} (Figure S7A). In dichloromethane solution, there are two (population relaxation) decay components of the azido stretching mode of PEGME−N_3, where the corresponding decay time constants are 1.4 and 6.3 ps (Figure 4a,b).[25] On the other hand, in the aqueous 50 wt % PEGDME solution, both the fast and slow time components become much faster (lower dash lines in Figure 4a,b). Noting that the azido group strongly interacts with a water molecule, our observation is additional evidence that the azido-group of PEGME−N_3 can be a good IR reporter about its local H-bonding environment.

In the case of the denaturizing osmolyte, urea, the vibrational lifetime of the azido stretching mode does not depend on the concentration of urea (up to 4 M), which corroborates with our

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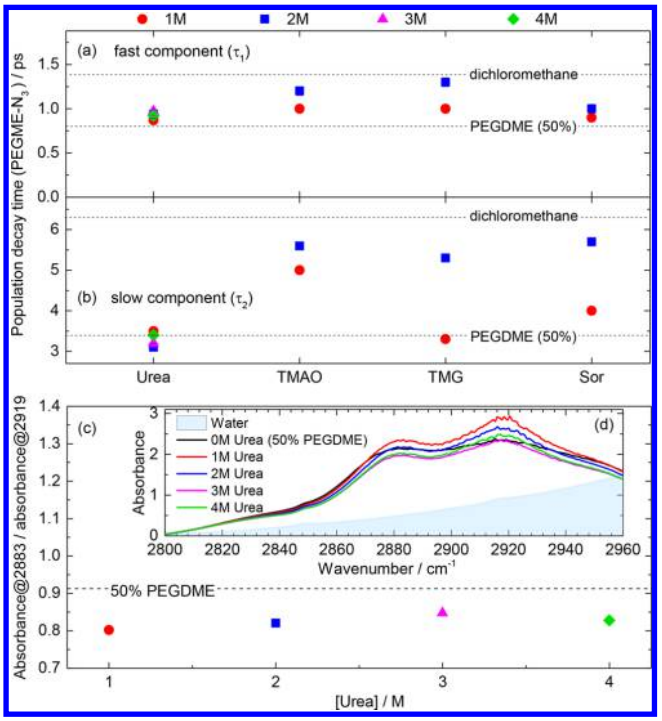

Figure 4. Vibrationally excited state of the asymmetric azido stretching mode of PEGME–N_3 decays biexponentially (τ_1 and τ_2) in dichloromethane as well as in osmolyte solutions with 50 wt % PEGDME. As protecting osmolyte concentration increases, both fast and slow components decay similar to those in dichloromethane. The ratios of the C–H stretching mode absorbance at 2883 cm^{-1} to that at 2919 cm^{-1} are plotted in panel c, whereas the C–H stretch IR spectra are shown in panel d. The peak at around 2919 cm^{-1} corresponds to the C–H stretching vibrations of PEGDME in random-coil structures, whereas that at around 2883 cm^{-1} results from the C–H stretching vibrations of PEGDME in both random-coil and *tgt* (trans–gauche–trans) helical conformations.

suggestion that surface hydration region of PEGDME remains unaffected by the presence of dissolved urea molecules because it does not cause any significant perturbation to water H-bonding network.

In contrast with urea, protecting osmolytes slow down the vibrational energy relaxation of the azido stretching mode of PEGME–N_3. The corresponding vibrational lifetimes in 2 M protecting osmolyte and 50 wt % PEGDME solutions are fairly close to those of PEGME–N_3 in dichloromethane. This means that the extent of surface hydration around each PEGDME is reduced by the dissolved protecting osmolyte molecules, which could be an important clue about the conformational transition of PEGDME upon dehydration induced by added protecting osmolyte molecules. Since each urea molecule fits very well in a given water H-bonding network, the dissolved urea molecules keep the PEGDME chain extended and well-hydrated, which makes random-coil structures of PEGDME stable and favored over helical conformations. However, protecting osmolytes induce a notable shift of the equilibrium toward the less hydrated state favoring helical confirmation. In the 50 wt % PEGDME solutions with protecting osmolytes, osmolyte-induced dehydration therefore promotes intramolecular interactions between segments in each individual PEGDME, which makes the relative population of helical segments increase. This is consistent with the fact that macromolecular association constants under crowded conditions increase as much as 2–3 orders of magnitude relative to those in the dilute solution.[43]

Urea-Induced Conformational Transition of PEGDME. The conformational transition of PEGDME segments can be experimentally estimated by comparing the C–H stretching vibrations of PEGDME at ~2919 cm^{-1} (associated with random-coil configuration of PEGDME) and ~2883 cm^{-1} (both random-coil and helical conformations of PEGDME).[39,40] Since urea does not have any vibrational modes that absorb IR fields in the frequency range from 2800 to 2960 cm^{-1}, the ratio of the IR absorbance at 2883 to that at 2919 cm^{-1} provides direct information on the PEGDME conformational propensity. Interestingly, this absorbance ratio decreases with the addition of urea (Figure 4c,d), which indicates a slight increase in the population of random-coil segments. This can be understood by noting that urea molecules are capable of forming ideal H-bonding interactions with other water molecules.

Biological Implications. On the basis of the present experimental results, we would like to discuss how osmolyte molecules might operate in a biologically crowded environment? As mentioned earlier, there exist two different viewpoints in many studies about its mode of action, i.e., direct and indirect mechanisms. Urea, one of the most potent denaturants, remains a neutral spectator in aqueous solutions. It neither weakens nor strengthens the H-bonding network of water.[19] Our experimental data thus rules out urea being considered as a water structure breaker. Urea solvates macromolecule PEGDME equally well like water, and the surface hydration shell of the macromolecule remains well hydrated even at a high concentration of urea (4 M). The favorable interaction between urea and PEGDME makes the macromolecule adopt random coil segments over the helical structure. Although the macromolecule's backbone unit, which is O–CH_2–CH_2–O in the case of PEGDME differs from the peptide bond in proteins, they share some commonality like presences of hydrophobic and hydrophilic segments and relatively free dihedral rotational degrees of freedom both in PEGDME and protein. Our experimental results support the "direct" mechanism based on urea's tendency to accumulate around both the protein backbone and side chains, owing, for example, to strong dispersion interactions between urea molecules and protein backbone moieties that preferentially stabilize the unfolded state as suggested by previous studies.[7−12]

All protecting osmolyte molecules studied in the present study, however, prefer to make strong osmolyte–water H-bonding interactions. This leads to a decrease in the activity of water in solution. Water's fluctuating H-bonding network always competes, whenever possible, with protein's intrinsic H-bonding partners to form additional H-bonds with, for example, protein backbone peptides. Any decreased strength and number of water H-bonds with the protein would destabilize the unfolded state of proteins, where more peptide units are exposed to solvent. This is to say, protecting osmolytes can enhance intraprotein interactions by reducing the number of available water molecules that are competing for hydration sites at the protein. The direct evidence of this indirect mechanism is confirmed by our experimental results indicating a certain degree of dehydration of macromolecule by protecting osmolyte molecules in macromolecular crowding solutions.

Here, our dual-IR-probe approach with femtosecond polarization-selective IR PP measurement method has been shown to be useful to elucidate the underlying mechanism of

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osmolyte's operation on a simple macromolecule (polymer). The vibrational population relaxation of the OD−e water in the presence of urea is similar to that in 50 wt % PEGDME solution with no urea. Also, the vibrational lifetime of the azido stretching mode of PEGME−N_3 is independent of the concentration of urea. These two observations provide evidence that urea molecules can interact with the macromolecule, which in turn suggests that urea's ability to denature proteins does not arise from its indirect effect on the structure of water but from its more direct interactions with proteins. Unlike urea, protecting osmolytes, TMAO, TMG, and sorbitol, slow down the vibrational energy relaxation of the OD stretching mode in the OD−e species, as compared to that in 50 wt % PEGDME solution with no protecting osmolyte. In addition, the vibrational lifetimes of the azido stretching mode of PEGME−N_3 in 2 M protecting osmolyte solutions are close to those in dichloromethane solution. This is important evidence that protecting osmolytes can enhance protein stability by attenuating the strength of the H-bonds formed between protein polar groups and water. It will be interesting to extend our dual IR probe approach to systems of increasing complexity like proteins to shed light on the role of osmolyte molecules in protein conformations.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpclett.7b03297.

Experimental section, FTIR spectra of the OD stretching mode of HDO and the azido stretching mode of PEGME−N_3 in 50 wt % PEGDME + osmolyte solutions, peak frequencies of the OD stretching mode in osmolyte water solutions, IR PP spectra of the OD stretching mode of HDO and the azido stretching mode of PEGME−N_3 in 50 wt % PEGDME + osmolyte solutions, fitting analyses of the FTIR and IR PP spectra, heat correction procedure, vibrational lifetimes of the OD stretching mode of HDO in osmolyte water solutions, and IR PP anisotropic data (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Yancey, P.; Clark, M.; Hand, S.; Bowlus, R.; Somero, G. Living with Water Stress: Evolution of Osmolyte Systems. *Science* **1982**, *217*, 1214−1222.

(2) Yancey, P. H. Water Stress, Osmolytes and Proteins. *Am. Zool.* **2001**, *41*, 699−709.

(3) Nandi, P. K.; Bera, A.; Sizaret, P. Y. Osmolyte Trimethylamine N-Oxide Converts Recombinant α-Helical Prion Protein to its Soluble β-Structured Form at High Temperature. *J. Mol. Biol.* **2006**, *362*, 810−820.

(4) Scaramozzino, F.; Peterson, D. W.; Farmer, P.; Gerig, J. T.; Graves, D. J.; Lew, J. TMAO Promotes Fibrillization and Microtubule Assembly Activity in the C-Terminal Repeat Region of Tau. *Biochemistry* **2006**, *45*, 3684−3691.

(5) Uversky, V. N.; Li, J.; Fink, A. L. Trimethylamine-N-oxide-induced folding of α-synuclein. *FEBS Lett.* **2001**, *509*, 31−35.

(6) Fallah, M. A.; Gerding, H. R.; Scheibe, C.; Drescher, M.; Karreman, C.; Schildknecht, S.; Leist, M.; Hauser, K. Simultaneous IR-Spectroscopic Observation of α-Synuclein, Lipids, and Solvent Reveals an Alternative Membrane-Induced Oligomerization Pathway. *ChemBioChem* **2017**, *18*, 2312−2316.

(7) Hua, L.; Zhou, R.; Thirumalai, D.; Berne, B. J. Urea Denaturation by Stronger Dispersion Interactions with Proteins than Water Implies a 2-stage Unfolding. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 16928−16933.

(8) Rezus, Y. L. A.; Bakker, H. J. Effect of Urea on the Structural Dynamics of Water. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 18417−18420.

(9) Canchi, D. R.; García, A. E. Backbone and Side-Chain Contributions in Protein Denaturation by Urea. *Biophys. J.* **2011**, *100*, 1526−1533.

(10) Tobi, D.; Elber, R.; Thirumalai, D. The Dominant Interaction between Peptide and Urea is Electrostatic in Nature: A Molecular Dynamics Simulation Study. *Biopolymers* **2003**, *68*, 359−369.

(11) Guinn, E. J.; Pegram, L. M.; Capp, M. W.; Pollock, M. N.; Record, M. T. Quantifying Why Urea is a Protein Denaturant, Whereas Glycine Betaine is a Protein Stabilizer. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*, 16932−16937.

(12) Canchi, D. R.; Paschek, D.; García, A. E. Equilibrium Study of Protein Denaturation by Urea. *J. Am. Chem. Soc.* **2010**, *132*, 2338−2344.

(13) Frank, H. S.; Franks, F. Structural Approach to the Solvent Power of Water for Hydrocarbons; Urea as a Structure Breaker. *J. Chem. Phys.* **1968**, *48*, 4746−4757.

(14) Bennion, B. J.; Daggett, V. Counteraction of Urea-induced Protein Denaturation by Trimethylamine N-oxide: A Chemical Chaperone at Atomic Resolution. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 6433−6438.

(15) Wei, H.; Fan, Y.; Gao, Y. Q. Effects of Urea, Tetramethyl Urea, and Trimethylamine N-Oxide on Aqueous Solution Structure and Solvation of Protein Backbones: A Molecular Dynamics Simulation Study. *J. Phys. Chem. B* **2010**, *114*, 557−568.

(16) Panuszko, A.; Bruździak, P.; Zielkiewicz, J.; Wyrzykowski, D.; Stangret, J. Effects of Urea and Trimethylamine-N-Oxide on the Properties of Water and the Secondary Structure of Hen Egg White Lysozyme. *J. Phys. Chem. B* **2009**, *113*, 14797−14809.

(17) Knake, L.; Schwaab, G.; Kartaschew, K.; Havenith, M. Solvation Dynamics of Trimethylamine N-Oxide in Aqueous Solution Probed by Terahertz Spectroscopy. *J. Phys. Chem. B* **2015**, *119*, 13842−13851.

(18) Luan, B.; Shan, B.; Baiz, C.; Tokmakoff, A.; Raleigh, D. P. Cooperative Cold Denaturation: The Case of the C-Terminal Domain of Ribosomal Protein L9. *Biochemistry* **2013**, *52*, 2402−2409.

(19) Verma, P. K.; Lee, H.; Park, J. Y.; Lim, J. H.; Maj, M.; Choi, J. H.; Kwak, K. W.; Cho, M. Modulation of the Hydrogen Bonding Structure of Water By Renal Osmolytes. *J. Phys. Chem. Lett.* **2015**, *6*, 2773−2779.

(20) Ahmed, M.; Namboodiri, V.; Mathi, P.; Singh, A. K.; Mondal, J. A. How Osmolyte and Denaturant Affect Water at the Air−Water Interface and in Bulk: A Heterodyne-Detected Vibrational Sum

DOI: 10.1021/acs.jpclett.7b03297
J. Phys. Chem. Lett. 2018, 9, 724−731

Frequency Generation (HD-VSFG) and Hydration Shell Spectroscopic Study. *J. Phys. Chem. C* **2016**, *120*, 10252−10260.
(21) Rezus, Y. L. A.; Bakker, H. J. Destabilization of the Hydrogen-Bond Structure of Water by the Osmolyte Trimethylamine N-Oxide. *J. Phys. Chem. B* **2009**, *113*, 4038−4044.
(22) Mondal, J. A. Effect of Trimethylamine N-Oxide on Interfacial Electrostatics at Phospholipid Monolayer−Water Interfaces and Its Relevance to Cardiovascular Disease. *J. Phys. Chem. Lett.* **2016**, *7*, 1704−1708.
(23) Ohto, T.; Backus, E. H. G.; Mizukami, W.; Hunger, J.; Bonn, M.; Nagata, Y. Unveiling the Amphiphilic Nature of TMAO by Vibrational Sum Frequency Generation Spectroscopy. *J. Phys. Chem. C* **2016**, *120*, 17435−17443.
(24) Phillip, Y.; Schreiber, G. Formation of Protein Complexes in Crowded Environments − From in Vitro to in Vivo. *FEBS Lett.* **2013**, *587*, 1046−1052.
(25) Verma, P. K.; Kundu, A.; Ha, J.-H.; Cho, M. Water Dynamics in Cytoplasm-Like Crowded Environment Correlates with the Conformational Transition of the Macromolecular Crowder. *J. Am. Chem. Soc.* **2016**, *138*, 16081−16088.
(26) Daley, K. R.; Kubarych, K. J. An "Iceberg" Coating Preserves Bulk Hydration Dynamics in Aqueous PEG Solutions. *J. Phys. Chem. B* **2017**, *121*, 10574−10582.
(27) Kjellander, R.; Florin, E. Water structure and changes in thermal stability of the system poly(ethylene oxide)-water. *J. Chem. Soc., Faraday Trans. 1* **1981**, *77*, 2053−2077.
(28) Rezus, Y. L. A.; Bakker, H. J. On the Orientational Relaxation of HDO in Liquid Water. *J. Chem. Phys.* **2005**, *123*, 114502.
(29) Steinel, T.; Asbury, J. B.; Zheng, J.; Fayer, M. D. Watching Hydrogen Bonds Break: A Transient Absorption Study of Water. *J. Phys. Chem. A* **2004**, *108*, 10957−10964.
(30) Kundu, A.; Błasiak, B.; Lim, J.-H.; Kwak, K.; Cho, M. Water Hydrogen-Bonding Network Structure and Dynamics at Phospholipid Multibilayer Surface: Femtosecond Mid-IR Pump−Probe Spectroscopy. *J. Phys. Chem. Lett.* **2016**, *7*, 741−745.
(31) Hunt, N. T. 2D-IR spectroscopy: ultrafast insights into biomolecule structure and function. *Chem. Soc. Rev.* **2009**, *38*, 1837−1848.
(32) Bakulin, A. A.; Cringus, D.; Pieniazek, P. A.; Skinner, J. L.; Jansen, T. L. C.; Pshenichnikov, M. S. Dynamics of Water Confined in Reversed Micelles: Multidimensional Vibrational Spectroscopy Study. *J. Phys. Chem. B* **2013**, *117*, 15545−15558.
(33) Wolfshorndl, M. P.; Baskin, R.; Dhawan, I.; Londergan, C. H. Covalently Bound Azido Groups Are Very Specific Water Sensors, Even in Hydrogen-Bonding Environments. *J. Phys. Chem. B* **2012**, *116*, 1172−1179.
(34) Kuzmin, A. V.; Neumann, C.; van Wilderen, L. J. G. W.; Shainyan, B. A.; Bredenbeck, J. Exploring photochemistry of p-bromophenylsulfonyl, p-tolylsulfonyl and methylsulfonyl azides by ultrafast UV-pump-IR-probe spectroscopy and computations. *Phys. Chem. Chem. Phys.* **2016**, *18*, 8662−8672.
(35) Tayama, J.; Ishihara, A.; Banno, M.; Ohta, K.; Saito, S.; Tominaga, K. Temperature dependence of vibrational frequency fluctuation of N3− in D2O. *J. Chem. Phys.* **2010**, *133*, 014505.
(36) Lee, H.; Choi, J.-H.; Verma, P. K.; Cho, M. Computational Vibrational Spectroscopy of HDO in Osmolyte−Water Solutions. *J. Phys. Chem. A* **2016**, *120*, 5874−5886.
(37) Lawrence, C. P.; Skinner, J. L. Vibrational Spectroscopy of HOD in Liquid D_2O. II. Infrared Line Shapes and Vibrational Stokes Shift. *J. Chem. Phys.* **2002**, *117*, 8847−8854.
(38) Oxtoby, D. W. Vibrational Relaxation in Liquids. *Annu. Rev. Phys. Chem.* **1981**, *32*, 77−101.
(39) Gemmei-Ide, M.; Miyashita, T.; Kagaya, S.; Kitano, H. Mid-infrared Spectroscopic Investigation of the Perfect Vitrification of Poly(ethylene glycol) Aqueous Solutions. *Langmuir* **2015**, *31*, 10881−7.
(40) Liu, K.-J.; Parsons, J. L. Solvent Effects on the Preferred Conformation of Poly(ethylene glycols). *Macromolecules* **1969**, *2*, 529−533.
(41) Laage, D.; Hynes, J. T. A Molecular Jump Mechanism of Water Reorientation. *Science* **2006**, *311*, 832−835.
(42) Lee, H.; Choi, J.-H.; Verma, P. K.; Cho, M. Spectral Graph Analyses of Water Hydrogen-Bonding Network and Osmolyte Aggregate Structures in Osmolyte−Water Solutions. *J. Phys. Chem. B* **2015**, *119*, 14402−14412.
(43) van den Berg, B.; Ellis, R. J.; Dobson, C. M. Effects of Macromolecular Crowding on Protein Folding and Aggregation. *EMBO J.* **1999**, *18*, 6927−6933.

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J. Phys. Chem. Lett. 2018, 9, 724−731