Hydrogen Bond Dynamics in Aqueous Solutions of Osmolytes Studied by Femtosecond IR Spectroscopy

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Abstract: Ultrafast hydrogen-bond dynamics in presence of osmolytes are studied by femtosecond IR spectroscopy using two different vibrational probes (HOD and HN_3). Dual-IR-probe approach enables us to have stereoscopic and complementary views on osmolyte-induced changes of water structure and dynamics.
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1. Introduction

Osmolytes are small naturally occurring molecules that are produced and accumulated in the intracellular environment at relatively high concentrations to maintain the intracellular proteins in a soluble form under the environmental stress such as extremes of temperature, pressure and solution composition [1, 2]. Human kidney is one such example of osmotic stress under normal conditions [3]. The mammalian kidney accumulates high concentrations of sorbitol, N,N,N-trimethylglycine (TMG) and glycerophosphocholine and uses them to counteract the deleterious effects of high renal urea and salt concentrations [4]. However, the mechanism of the osmolytes action is still under debate.

To be specific, the question that we ask is whether osmolytes modulate water H-bond properties or act directly on the proteins? If they do modulate the properties of water H-bonding network, does the presence of multiple H-bond donor and/or acceptor sites (OH or HN) in osmolytes play a critical role? To provide conclusive answers, we use femtosecond (fs) IR pump−probe (PP) and 2D IR measurements. Our approach is different from the previous works because of the use of two different IR probes. First one is the OD stretch mode of the HDO dissolved in osmolytes solution, which has been routinely used by many research groups because HDO can form an H-bond with water as well as osmolyte molecules [5-7]. However, HDO provides information on water structure only from the water's point of view and does not provide any information on how a given osmolyte dictates water's choice to be H-bonded either with another water or a third molecular component (e.g., protein or solute) in solution. To get information from the dissolved solute's point of view, we use another IR probe, hydrazoic acid (HN_3), which allows a direct assessment of how water's choice of H-bonding is altered by osmolyte−water and osmolyte−osmolyte interactions. Thus, our dual-IR-probe approach enables us to have stereoscopic and complementary views on osmolyte-induced changes of water structure.

2. Experiment

For IR PP measurements, we used a pump-probe geometry with collinear pump and probe pulses, all centered at either 2530 cm^{-1} or 2140 cm^{-1}. For 2D IR measurements, we used three independently controlled pulses (all centered at 2140 cm^{-1}) that focus into the sample in a "boxcar" geometry. The absorbances of both OD and NNN vibration bands were kept in the range from 0.2 to 0.4 for IR measurements by using a teflon spacer of appropriate thickness (6, 12 and 25 μm). All experiments were performed at room temperature.

3. Results and Discussion

In Fig. 1a-d, we present the peak maximum (amount as a shift in peak position) and FWHM (full width at half-maximum height) obtained from the fitting of the absorbance spectra of the OD and azide band at various concentrations of different osmolytes with a Voigt function. Although the line shapes are not perfect Voigt, the fits, nonetheless, reflect the changes with increasing osmolyte concentration. All the osmolytes except urea causes a red-shift of OD band with significant line-broadening observed on the red-side while weaker narrowing on the blue-side of the spectrum. The red-shift suggests an increase in the number or strengthening of H-bond network in osmolyte solutions [8]. Among all the studied osmolytes, the largest red-shift (17 cm^{-1}) is observed in sorbitol.

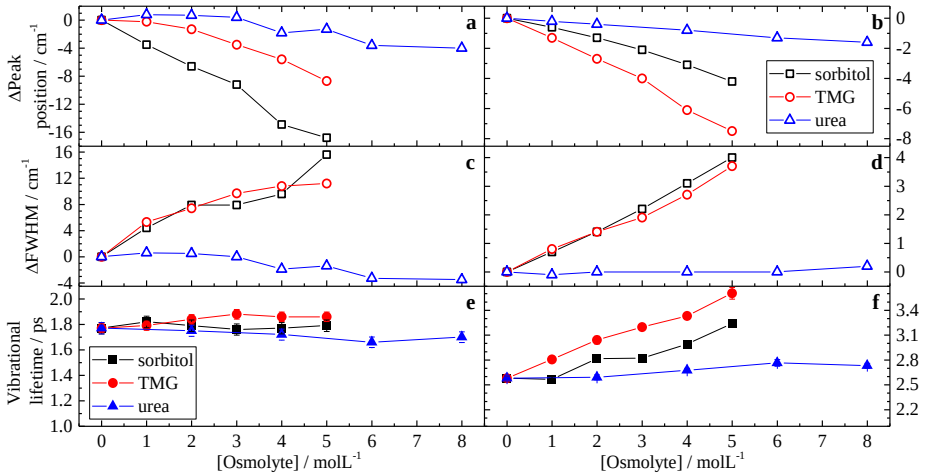

Fig. 1. ΔPeak position and ΔFWHM of Voigt fits to the FTIR spectra of the OD (a, c) and azide (b, d) bands as a function of osmolytes concentrations. Vibrational lifetime of the OD (e) and azide (f) bands as a function of various osmolytes concentrations.

Similar to OD band, azide band shows a substantial red-shift (Fig. 1b) on addition of osmolytes except urea. This shift is due to the change in the population of NNN-H_2O H-bond rather than its strength [9]. This red-shift provides direct indication that TMG, which by itself is unlikely to form direct H-bonds with the NNN group, can decrease the NNN-H_2O H-bond population while enhancing the TMG-H_2O at the expense of water-water. Although sorbitol causes a smaller red-shift of the azide band than that of OD band, the increase in FWHM is almost the same for both bands. This is due to the H-bond forming capacity of sorbitol. Sorbitol having six hydroxyl groups can form an H-bond with NNN apart from NNN-H_2O H-bonds, whereas, in the case of TMG (zwitterion) without OH, there exist mainly NNN-H_2O H-bonds. What is more interesting to note is that both the peak position and the FWHM of OD and azide bands are independent of the urea concentration. This supports the notion that urea neither changes the equilibrium number of H-bonds and H-bond strengths nor does it perturb the NNN-H_2O partnership, meaning that urea−water interactions are very well balanced with water-water and urea-urea interactions.

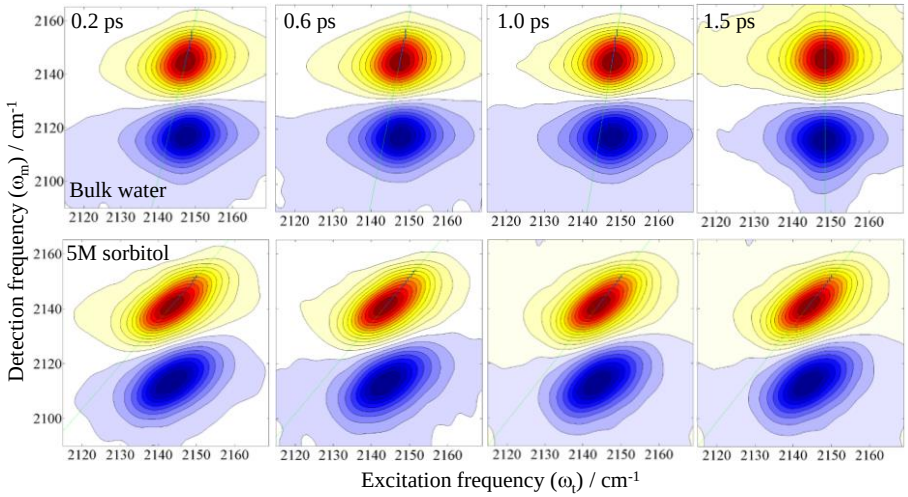

Fig. 2. 2D-IR spectra of azide band in bulk water (upper) and 5M sorbitol (lower) at different waiting times.

The vibrational lifetime of the OD and azide stretching modes as a function of various osmolyte concentrations are shown in Fig. 1e and f. The vibrational lifetime of the OD band shows negligible dependence on osmolyte concentration indicating that osmolytes have negligible effect on the vibrational relaxation of the OD stretch mode. Unlike OD, vibrational lifetime of azide band significantly increases with osmolytes (except urea) concentration, which reflects HN_3's unique sensitivity to local H-bonding interaction. The increase in the lifetime with increasing osmolyte concentration clearly indicates that the NNN-H_2O H-bond population is significantly reduced. The decreased availability of H-bonding partner towards HN_3 is manifested by its increased lifetime in the presence of osmolytes. At all concentrations, TMG is more effective in perturbing water-water H-bonding partnership compared

to sorbitol as seen by the increasing vibrational lifetime of the azide. Sorbitol with six available hydroxyl groups can form H-bonds among themselves as well as to other molecules present in the solution. Such diversity in H-bonding capability seems to compensate for the H-bonding partner and hence the increase of the vibrational lifetime of azide (in sorbitol solution) is small when compared to TMG at the same concentration. Adding osmolyte does not always mean to break/form H-bond or to change the solute-water population as seen in the case of urea. Urea seems to be very compatible with water in a sense that water finds both urea and water to be an equally suitable partner for H-bonding, even up to very high concentration (8 M).

Fig. 2 presents 2D IR spectra of HN_3 in bulk water and in 5 M sorbitol solution at different waiting times. At short times, the spectrum is elongated along the diagonal, which indicates a highly correlated response. With time, the memory for initial excitation frequency still exists, keeping the 2D signal slightly tilted. To quantify the phase memory, the center line slope (CLS) analysis was applied. CLS being proportional to FFCF (frequency–frequency correlation function) fully describe the spectral diffusion process [10, 11]. The retrieved correlation function of HN_3 in bulk water decays at a time scale of ~ 1.5 ps. Compared to bulk water, the time scales of the spectral diffusion increases to 4 and 6 fold in presence of 4 M TMG and 5 M sorbitol, respectively. This increase in the spectral diffusion time is much larger than the corresponding increase in the vibrational lifetime of HN_3 in TMG and sorbitol. Altogether, this suggests both sorbitol and TMG slow down the water dynamics by limiting the available H-bonding partners for fast hydrogen bond switching while inhibiting the required coordinated reorientation that accompanies large angular jump.

4. Conclusion

Here, we have demonstrated that the OD probe tells us a one-sided story from water's point of view and does not allow one to draw a complete picture on the general dissolved solute effects on H-bonding structure of water. Thus, the neutral HN_3 probe appears to be a highly promising probe for monitoring osmolyte-induced perturbation to water structure and dynamics even in systems comprising of other possible H-bonding groups.

4. References

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