

Effect of osmolyte on hydrogen bonding network of water molecules

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Abstract: Vibrational spectroscopy was used to probe osmolyte-induced perturbation to water structure and dynamics. The experiments were carried out on aqueous solutions of myo-inositol, sorbitol, taurine, and trimethylglycine covering a wide range of molar concentration using two different vibrational probes (OD stretch of HOD and hydrazoic acid). Azide (NNN) vibrational mode appears to be highly sensitive in reporting structural changes in local hydrogen bonding network of liquid water. The results of our measurement clearly show that protecting osmolytes do modulate the hydrogen bonding networking in water.

Key words: Denaturant, hydrogen bonding, mid-infrared pump-probe, osmolyte, vibrational lifetime.

1. INTRODUCTION

Osmolytes are small naturally occurring molecules that are produced and accumulated inside cells in high amount to counter the environmental stress such as extremes of temperature, pH, cellular dehydration, desiccation, high extracellular salt[1]. Human kidney is one such environment where various osmolytes such as myo-inositol, taurine, trimethylglycine (TMG), and sorbitol are used to counteract the deleterious effects of high renal urea and salt. However, the mechanism of the osmolytes action is still under debate. One school of thought is direct mechanism where the osmolytes interact directly with the protein[2]. While, others believe that osmolytes act indirectly through the surrounding water, modifying its properties[3].

In this paper we present results of studies on protein-free binary solution of osmolytes and water.

2. RESULTS

The OD spectrum of HOD in water is very broad as shown in Figure 1. The broad line width arises due to a wide range of hydrogen bond strengths and different number of hydrogen bonds[4]. Species with stronger and/or more hydrogen bonds absorb on the red side (low-frequency region) of the spectrum while those with weaker and/or fewer absorb on blue side (high-frequency region) of the spectrum. Addition of sorbitol significantly shifts the peak position to low-frequency region (Figure 1) suggesting an increase in the equilibrium number of hydrogen bonds and/or an increase in the average hydrogen bonding strength. Similar behavior is observed with addition of TMG in isotopically diluted water.

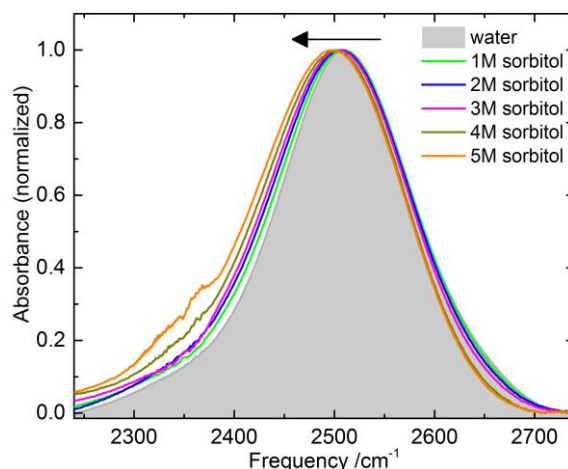


Figure 1. FTIR spectra of the OD stretch of HOD and in aqueous sorbitol. The concentrations are shown in molar (molL^{-1}) scale.

Hydrazoic acid (HN_3) is a small organic azido compound whose NNN stretching mode has a unique sense of hydration. Its stretching frequency shows no remarkable dependency on solvent polarity but is highly correlated to the number and orientation of H-bond donors[5]. Therefore, HN_3 provides a promising model system to probe whether the addition of a third molecular component (here osmolytes) will affect the NNN- H_2O hydrogen bond. The NNN band shows substantial red-shift upon addition of sorbitol (Figure 2) indicating a significant modification in the hydrogen bonding structure of the binary mixture. This decrease in the NNN stretching frequency provides direct indication that, the addition of sorbitol decreases the solute-water (NNN- H_2O) hydrogen bonding interaction while enhancing the sorbitol-water and/or sorbitol-NNN partnership at the expense of water-water. In case of TMG, the magnitude of peak shift is larger (7 cm^{-1})

compared to that of sorbitol (4 cm^{-1}).

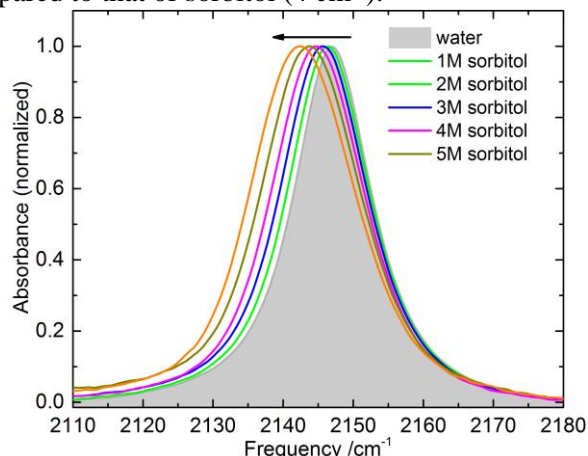


Figure 2. FTIR spectra of the azide stretch of hydrazoic acid in aqueous sorbitol. The concentrations are shown in molar (molL^{-1}) scale.

In Figure 3. We present two representative isotropic pump-probe signal of OD and azide stretching mode for 4 molar sorbitol solution. From the fitting of IR pump-probe signal, we obtain a value of 1.8 ps for the vibrational lifetime of OD stretch which is in excellent agreement with the previous measurements[6]. The time constants show negligible dependence on osmolyte concentration indicating that osmolytes have negligible effect on the vibrational relaxation of the OD stretch mode.

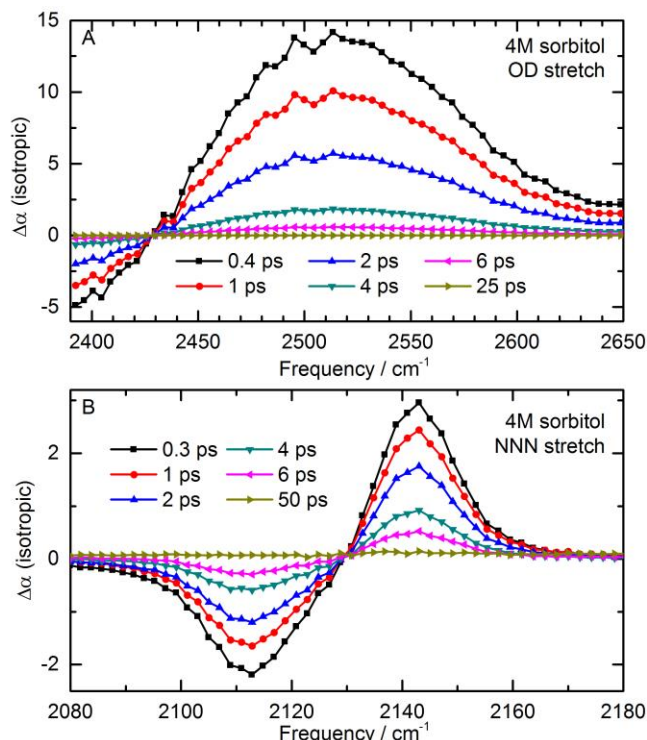


Figure 3. Isotropic IR pump-probe signals of sorbitol using OD stretch (A) and HN_3 stretch vibration (B).

On the other hand, addition of osmolytes to the aqueous solution of HN_3 induces a substantial increase in the vibrational lifetime of azide stretch mode. The extent of the perturbation is large in TMG while moderate in case of sorbitol. Main results will be published elsewhere.

3. CONCLUSION

Among all the renal osmolytes investigated in the present study, TMG appears to be highly effective in modulating water structure and dynamics. TMG is found to reduce the preference of water towards solute while strengthening the TMG-water population at the expense of water-water population. Such a behaviour is expected to strengthen the intra-protein hydrogen bond and hence increase the stability of the folded state of a protein. Sorbitol behaves similar to TMG but its impact on the hydrogen bonding network seems to be more diverse and long ranged. The reason is the availability of 6 hydroxyl units from sorbitol which provides hydrogen bond forming capability among themselves while keeping a considerable population of water-water intact. The limited solubility of taurine and myo-inositol restricts their studied concentration range. However, their behaviour also falls in the line of sorbitol and TMG.

REFERENCES

- [1] Yancey PH, et al. *Science* **217**, 1214 (1982)
- [2] Auton M, et al. *Proteins: Struct., Funct., Bioinf.* **73**, 802 (2008)
- [3] Roche CJ, et al. *J. Biol. Chem.* **281**, 38757 (2006)
- [4] Lawrence CP, et al. *J. Chem. Phys.* **117**, 8847 (2002)
- [5] Wolfshorndl MP, et al. *J. Phys. Chem. B* **116**, 1172 (2012)
- [6] Moilanen DE, et al. *J. Phys. Chem. B* **113**, 8560 (2009)