Urea and Guanidinium Chloride Act as 'Water Structure Breakers': the Debate Revisited by Dielectric Relaxation Study in THz Range

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***Abstract*— We have addressed a very fundamental question, whether urea and guanidinium chloride (GdmCl) act as water 'structure breakers'. Our study using THz time domain spectroscopy (TTDS) confirm that both these molecules perturb the extended solvation layer around themselves which supports the strongly debated "water structure breaker" notion of urea and GdmCl during their protein denaturation process[1,2].**

I. INTRODUCTION

THE unique ability of urea and guanidinium hydrochloride (GdmCl) to denature most of the proteins is a very established and yet very debated issue in biophysics. It is believed either to result from their direct interaction with the protein backbone or as an indirect effect in which urea perturbs the water network structure[3]. The former argument has been supported by simulation and experiments; however, the later hypothesis has been debated in the past. While most of the earlier studies involved the dynamics of water limited to first 1-2 hydration layer of the salts, the cooperative H-bond network dynamics which extends up to 3-4 hydration layer and leaves its imprint in the elusive THz frequency region[4], has never been investigated. We have made an attempt to understand the ultrafast solvation dynamics of water around these molecules by using a triple Debye dielectric relaxation model in the THz (0.3-2.0 THz) frequency region using THz time domain spectroscopy (TTDS). The principal motive of the present investigation is to underline the effect of these salts on the collective hydration dynamics of water. We also investigate comparable molecules like trimethylamine-N-oxide (TMAO) to render further support to our investigations. To address the changes in the hydration of protein we investigate the THz response of a globular protein human serum albumin (HSA) in presence of the denaturing agent GdmCl.

II. EXPERIMENTS & RESULTS

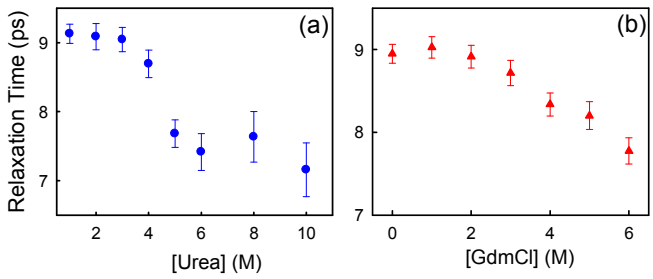

Fig.1. Co-operative relaxation timescale of water in presence of urea (a) and GdmCl (b) at different concentrations.

The THz measurements were carried out in a commercial THz spectrometer (Tera K8, Menlo Systems) using a 780 nm Er doped fiber laser having pulse width of <100 fs and a repetition rate of 100 MHz which excites a THz emitter antenna (gold dipoles deposited on LTG-GaAs substrate with a dipole gap of 5 μm) producing THz radiation having a bandwidth up to 3.0 THz (> 60 dB)[5]. The THz spectra in air is found to be fairly smooth with a high signal to noise ratio and minimal water absorption lines up to ~2.5 THz which enables us to extract the optical parameters with high precision up to ~2 THz.

We obtain the THz response of pure water and solutions of urea and GdmCl (obtained from Sigma Aldrich, of the highest available purity) in the frequency range of 0.2-3.0 THz. From the extracted absorption coefficient, $\alpha(\nu)$ and complex refractive index $n(\nu)$, we obtain the frequency dependent real and imaginary dielectric constants $\varepsilon(\omega)$, which we then fit to a triple Debye relaxation model,

$$\tilde{\varepsilon}(\omega) = \varepsilon_\infty + \sum_{j=1}^{3} \frac{S_j}{1+i\omega\tau_j} + \frac{\sigma}{i\omega\varepsilon_0}$$

where ε_∞ is the extrapolated dielectric constant at a very high frequency, S_j is the relaxation strength of the j-th relaxation mode and τ_j is the relaxation time of the corresponding mode, σ is the dc conductivity of the solution and ε_0 is the permittivity in free space. The last term appears for electrolyte solutions (herein GdmCl). The Debye relaxation produces three timescales of ~9 ps, ~200 fs and ~80 fs for pure water[1,2]. The 9 ps timescale is associated to the well known cooperative rearrangement of the hydrogen bonded network in water while the other two faster timescales are more related to the rotational modes of individual water molecules. The cooperative relaxation time of water defines the ultrafast collective network of water molecules in the extended hydration sheathe which extends up to 3-4 hydration layers.

We initiate our study with a rather indifferent solute sucrose to understand the dynamics of water around its surface. It is observed that with increase in sucrose concentration, the relaxation dynamics becomes slower, cf. at 1.6 M sucrose the timescale becomes ~ 16 ps[1]. This observation clearly identifies the formation of highly surface bound water network around sucrose and supports the notion of 'structure maker' for this solute. On the other hand an altogether different phenomenon emerges in case of urea and GdmCl. We measure the co-operative relaxation time for urea solutions of different concentrations and the timescales are plotted in figure 1a as a function of the salt concentration. It can be observed that for urea the cooperative dynamics (τ_1) suffers only marginal change up to 4 M beyond which it appreciably decreases to a low value of 7.1 ps. This drastic decrease in τ_1 unambiguously points out towards a faster cooperative hydrogen bond

rearrangement dynamics, which could have originated from a possible disruption in the extended hydration layer around urea. A similar change has also been observed in the τ_2 values wherein the timescale decreases sharply in the 4-6 M urea region [1]. It is also interesting to note that the water structure perturbation occurs in the 4-6 M urea which interestingly coincides with the threshold denaturing concentration of urea for most of the proteins. This leads us to conclude that the notion of 'structure breaking' is related to the unique property of urea to denature protein through an 'indirect mechanism'. Similar retarded dynamics has also been observed for the other two derivatives of urea, namely, thiourea and tetramethyl urea. We further investigate the hydration dynamics of another conventional structure maker molecule TMAO, and found that the overwhelming protein stabilizing ability of TMAO in presence of urea is also manifested in the hydration dynamics [1].

A similar retardation of dynamics is also observed in case of GdmCl (figure 1b) wherein it is observed that τ_1 does not change appreciably up to 2 M GdmCl, beyond which it decreases linearly to reach 7.7 ps at 6 M. Such an abrupt acceleration in the H-bond dynamics also manifests the 'structure breaking' notion of this salt. To understand the influence of the cation on the structure breaking effect, we consider two other salts having the same anion (Cl^-) and with two different cations: Na^+ and a more hydrophobic counterpart of guanidinium, $TMGdm^+$. It is observed that while Na^+ produces a linear acceleration of the dynamics, $TMGdm^+$, on the other hand, offer a linear retardation in the dynamics [2]. The 'structure breaking' ability of Na^+ is found to be more prominent than GdmCl and also unlike the later it is not concentration dependent. It can be noted that like in urea, GdmCl also shows the characteristic accelerated H-bond dynamics beyond a threshold salt concentration strikingly similar to the protein denaturation threshold of GdmCl.

We have also studied the fate of the dynamics of the hydration layer associated with proteins; we use a well characterized globular protein, human serum albumin (HSA) in presence of various salts. The protein is indifferent to NaCl, however, its tertiary structure suffers considerable perturbation in presence of GdmCl, specially beyond a concentration of 2M as evidenced from circular dichriosm (CD) measurements. We also measure the absorption coefficient $\alpha(\nu)$ of the protein solution in absence and in presence of GdmCl and NaCl. We observe two opposing trends in these two salts; in NaCl, the changes observed in $\Delta\alpha$ can be explained by safely considering additive contributions from the salt and the protein taken separately. However, in GdmCl, the additive rule does not hold good, and the observed trend in $\Delta\alpha$ can only be explained by considering a direct interaction between the protein and the salt[2].

III. SUMMARY

We have measured the various optical parameters of urea and GdmCl aqueous solutions in the 0.3-2.0 THz frequency range and fitted the real and imaginary parts of the complex dielectric constants in a triple Debye relaxation model. It was found that the cooperative relaxation dynamics of water gets faster beyond a 'threshold' salt concentration indicating towards a possible rupture of the collective hydrogen bond network of water. It is intriguing that the observed collapse occurs at a certain salt concentration which strikingly coincides with the denaturation concentration of the corresponding salts for many proteins. Despite the fact that TTDS does not provide any direct information on the structure of the solvents and thus not directly supports the notion of urea or GdmCl being a 'structure breaker', the extracted dynamics essentially hints towards a perturbation in the extended hydrogen bonded network in water, and also possibly invokes the idea that the 'indirect' mechanism of water structure breaking has non-negligible role to play in the protein denaturation process.

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