UREA-WATER DYNAMICS IN PROTEINS: AN ULTRAFAST SPECTROSCOPIC STUDY

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Water plays a vital role in many biological processes like enzyme activity, protein folding-refolding and denaturation. Interfacial water has a significant effect on the protein's internal structure and dynamics. It is essential to know the time scales characteristic of both, the local protein rearrangements and water dynamics within the solvation shell to understand the protein-water interactions. Urea is a chaotropic agent and a well-known denaturant for proteins. The molecular picture of the interaction of urea with the water hydrogen bond network and thereby, the chemical denaturation of the proteins is still ambiguous. Time-resolved Optical Kerr effect (OKE) spectroscopy is a powerful spectroscopic technique to study the hydrogen-bonded structure and dynamics of complex aqueous systems, in the picosecond time scales. In this study, we have investigated the mechanism behind urea denaturation of three proteins of different hydrophobicities- lysozyme, BSA and trypsin. The OKE data reveals the effect that different concentrations of urea have on the aqueous protein solutions. The temporal profiles of the aqueous protein solutions are almost indistinguishable from that of pure water. Resolving the data in the frequency domain and subtracting the solvent contribution gives us a better picture of the water and water-urea interactions with the protein. The spectral density (SD) obtained contains the α relaxation at the lowest frequencies corresponding to the orientational diffusion of the molecules, linked by the stretched β relaxation to the intermolecular librational modes at terahertz frequencies. The shape of the SDs resembles that of urea solutions; the addition of protein brings down the contribution from the α relaxation. At lower urea concentrations, this change is even more apparent. The OKE spectra of urea have a huge α contribution compared to that of water which masks the faster β dynamics. Removing the diffusive contributions in the time domain itself leaves us with the Reduced Spectral Densities (RSD). Preliminary analysis of the RSDs shows the β relaxation timescales of water changes on the addition of urea and the subtracted spectra for the urea denatured lysozyme shows two distinct β processes characteristic of water and water-urea dynamics. A detailed analysis of the changes in the line shapes of the RSDs is required to elucidate the effect urea has on the water hydrogen bond network and to map out the structural changes occurring in three different proteins on the addition of urea.