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Preferential solvation of lysozyme in dimethyl sulfoxide/water binary mixture probed by terahertz spectroscopy

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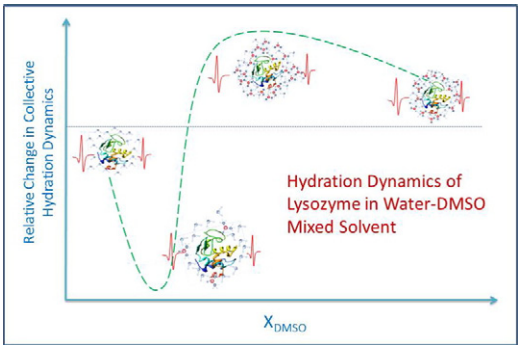
HIGHLIGHTS

- Addition of DMSO in lysozyme aqueous solution induces noticeable conformational changes.
- DMSO gets preferentially absorbed at the protein surface leading to the conformational changes.
- Collective hydration dynamics at the protein surface follows the trace of DMSO induced conformational changes.

GRAPHICAL ABSTRACT



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ABSTRACT

We report the changes in the hydration dynamics around a model protein hen egg white lysozyme (HEWL) in water–dimethyl sulfoxide (DMSO) binary mixture using THz time domain spectroscopy (TTDS) technique. DMSO molecules get preferentially solvated at the protein surface, as indicated by circular dichroism (CD) and Fourier transform infrared (FTIR) study in the mid-infrared region, resulting in a conformational change in the protein, which consequently modifies the associated hydration dynamics. As a control we also study the collective hydration dynamics of water–DMSO binary mixture and it is found that it follows a non-ideal behavior owing to the formation of DMSO–water clusters. It is observed that the cooperative dynamics of water at the protein surface does follow the DMSO-mediated conformational modulation of the protein.

1. Introduction

Aqueous environment of biomolecules often plays the key roles in both inter and intra-molecular interactions which are responsible for their stabilization; such interactions include hydrogen bonding, hydrophobic interactions, ionic bonding etc. [1,2] Addition of otherwise indifferent components like sugar, salts, organic solvents etc. can influence protein conformation [3] either by direct interaction or through preferential solvation, in which the additive preferentially or selectively exists in the protein solvation shell [4,5]. Owing to this unique ability binary solvents can control as well as tune the structure of protein or bio-molecules, and thus have received considerable attention in recent years.

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Water–dimethyl sulfoxide (DMSO) binary mixture has been found to be of potential interest as it plays a significant role in the field of chemistry, physics, biology and pharmacology [6–10]. Owing to the specific hydrogen bonding ability of DMSO the binary mixture often exhibits some unique physicochemical behavior [8]. DMSO acts as a protein stabilizer at low concentration whereas it destabilized proteins at high concentration which makes this binary mixture to be used as a stabilizer, a denaturant, an activator, or an inhibitor [11–14].

The effect of this binary mixture on the conformational stability of a model protein lysozyme has previously been studied by several research groups. Far-UV CD measurements concluded a native to unfolded structural transition of lysozyme at low DMSO concentration (X_{DMSO} ~ 0.06) [15]. At higher DMSO concentration the protein undergoes broad structural transition. NMR relaxation study by Johannesson et al. [14] has established a direct interaction of DMSO molecules with the active cleft of lysozyme. Kamiyama et al. [16] made a detailed thermodynamic study to establish a preferential solvation of lysozyme by DMSO in the binary mixture. Kovrigin et al. [17] have carried out a comparative study on the preferential solvation of DMSO and four other organic solvents during thermal denaturation of lysozyme and concluded that the initial interaction of the exposed groups of the denatured protein and organic solvent is non-specific in nature. Voets et al. [11] used small angle neutron scattering (SANS) technique to identify unfolded to partially collapsed denatured state of lysozyme with increasing DMSO concentration. A recent Molecular Dynamics (MD) simulation study by Roy et al. [18] has concluded the protein to pass through several conformational states as the composition of the mixture changes. Fluorescence correlation spectroscopy study by Ghosh et al. [19] by labeling lysozyme with Alexa 488 concluded the hydrodynamic radius of the protein to undergo systematic changes in the low DMSO concentration region (<30 mol%). All these above mentioned studies have unambiguously concluded substantial effect of DMSO on the protein conformation, however, less attention has been paid on the dynamics of the hydration layer associated with the protein as it undergoes conformational changes. In this contribution we probe the hydration dynamics of hen egg white lysozyme (HEWL) at different compositions of the DMSO–water binary mixture, mainly in the range of 0–0.5 mol fraction of DMSO, using THz time domain spectroscopy (TTDS) technique.

THz spectroscopy has recently emerged as a potential tool to label free determination of the collective hydrogen bond dynamics which extends up to several hydration layers [20] around solutes [21,22], also applicable for measuring the collective hydration dynamics at various compositions of binary mixtures [23] and biomolecules [24,25]. This technique offers a unique advantage to examine the fate of the H-bonded network dynamics of water in otherwise less polar environments including biological interfaces [26,27]. Various frequency-dependent optical parameters of the solution (viz. absorption coefficient, $\alpha(\nu)$, complex refractive index, $n \sim(\nu)$, complex dielectric constants, $\varepsilon\sim(\nu)$) etc. can be extracted from a single measurement [28]. In this frequency region it is possible to locate the low frequency collective modes that are responsible for the direct flow of conformational energy (as a result of the changes of interaction pattern with varying the concentration of protein in buffer medium) in many biological processes [25]. There have been a few earlier studies on the collective dynamics in partially or fully hydrated lysozyme using THz spectroscopy [29–31], however, a detailed understanding on the changes in the hydration dynamics in its different conformational states has not yet been attempted. We determine the changes in the tertiary structure of the protein in presence of DMSO using CD spectroscopy in the near-UV region. We obtain the hydrogen bonded structure of water by measuring the O-H bond stretch in the mid-IR region using FTIR technique. We investigate the hydration dynamics around HEWL at different compositions of the binary mixture using TTDS. The primary focus of this study is to understand whether the perturbation experienced by the protein in DMSO–water mixture has its imprint on the associated hydration structure of the protein and how the preferential solvation of protein by DMSO associates the altered dynamics.

2. Materials and methods

Hen egg white lysozyme (HEWL, crystallized lyophilized powder) and dimethyl sulfoxide (DMSO, HPLC grade) were procured from Sigma-Aldrich and were used as received. 10 mg ml^{-1} aqueous-HEWL solution was prepared in Milli-Q water. This low concentration of HEWL was used in order to avoid any clustering [32]. Milli-Q water was used for all the binary mixture preparation. In all the measurements only freshly prepared aqueous-HEWL–DMSO solutions were used.

FTIR spectra of water-DMSO and aqueous-HEWL-DMSO solutions were measured using FTIR 6300 JASCO spectrometer. The O-H stretch of HOD was measured in a solution of 4% D_2O in water. A 15 μm spacer was used for all the FTIR measurements. Circular dichroism (CD) measurements in the near-UV region were performed in a JASCO J-815 spectrometer using 1 mm cuvette. Due to the strong absorption of DMSO in the ~250 nm wavelength region, we do not perform the CD experiments in the far-UV region. The sample scan speed was kept at 50 nm min^{-1} with response time of 2 s. Three CD spectra were recorded in continuous mode and averaged for each CD experiment.

THz-time domain spectroscopy (TTDS) measurements were carried out in a commercial THz spectrophotometer (TERA K8, Menlo Systems) [33]. In brief, a 780 nm Er doped fiber laser (< 100 fs pulse width (FWHM), 100 MHz repetition rate) is split into pump and probe beams of equal power (~10 mW) using a polarizing beam splitter. The pump beam excites the THz emitter antenna producing a THz radiation having a bandwidth of ~3.0 THz (>60 dB). This THz radiation after transmitting through the sample is focused on a THz detector antenna which is gated by the probe laser beam. The THz antennas are gold dipoles with a dipole gap of 5 μm deposited on LTG–Ga As substrate. To avoid water vapor absorption, all the measurements were carried out in dry nitrogen atmosphere with a controlled humidity of <10% in a liquid cell (Bruker, model A-145) using z-cut quartz windows and Teflon spacer of 100 μm thickness. The samples were reloaded for five times in the sample cell and nine full scans were averaged together to minimize the error in the results. By varying the time delay between the probe and the pump beam the amplitude and phase of the THz electric field were measured as a function of time. The frequency dependent power and phase of the transmitted pulse is obtained using Fourier analysis of the measured electric field amplitude $E_{THz}(t)$. TTDS involves coherent detection mechanism and can thus measure both the amplitude and the phase of radiation in a single measurement and can provide with information on frequency dependent optical parameters of the system. Subsequently, the frequency dependent absorption coefficient $\alpha(\nu)$ (power attenuation) and index of refraction $n(\nu)$ (delay of the THz pulse) can be obtained.

3. Results and discussions

3.1. Circular dichroism (CD) studies

Fig. 1 represents the effect of DMSO on the protein's tertiary structure as extracted from the near-UV CD spectra. The peaks observed at ~287 and 292 nm (Fig. 1a) are due to the transitions of the tryptophan residues of lysozyme [34] and thus correspond to the tertiary structure of the protein. The intensity of the CD spectrum initially increases with increasing X_{DMSO} to reach a maximum at X_{DMSO} ~ 0.25 beyond which it decreases. Fig. 1b shows the DMSO induced variation in the CD signal intensity at 287 and 292 nm. In the low DMSO concentration region the spectral pattern remains almost unchanged where DMSO acts as a protein stabilizer. Previously reported fluorescence studies of the intrinsic tryptophan also concluded a marked blue shift of the emission maximum pointing towards a stabilization of the protein [16,19]. Earlier studies have confirmed that the changes in the CD signals vis-à-vis the