Accelerated Terahertz Water Dynamics under Osmotic Interaction of Lipid Bilayers and Polyethylene Glycol

Kihoon Eom[1,2], Heyjin Son [1,2], Jin-Young Jeong [1,2], Jungmin Jang [1,2] , In-Kyung Park[1,2], Seonmyeong Kim[1,2] and Gun-Sik Park[1,2]
[1]Department of Physics and Astronomy, Seoul National University, Seoul, 151-747, Korea
[2]Center for THz-Bio Application Systems, Seoul National University, Seoul, 151-747, Korea

***Abstract* — Intracelluar water is expected to have different physical properties from those of bulk water. Using polyethylene glycol, we induce model intracellular environment on DMPC lipid solution and we shows the accelerated movement of non-hydrogen bonding (NHB) water molecules interacting with DMPC in polyethylene glycol (PEG) solution from the dielectric response in the terahertz range. With the measurement in the GHz range, detailed behavior of hydration water is also analyzed.**

I. INTRODUCTION

It is well known that living cells are crowded with bio-molecules of proteins, DNA, membrane lipids, etc. The density of intracellular environment is estimated to be 80-400 mg/ml that is much higher than those for the in vitro experiments [1]. In this environment, the excluded volume effects reduce a reaction volume and surface area of the biomolecules that make fundamental difference from those in a dilute solution [1]. In addition, the solvent properties of intracellular water are different from those of a dilute solution. The water molecules in the vicinity of the biomolecules form layers of perturbed water, so-called 'hydration water'. Since the cellular components occupy 5-40% of cellular volume, the most of the intracellular water becomes hydration water and it increases the amount of osmotically inactive bound water [2]. The amount of free water inside the cell changes depending on the status of the cell. For example the growth rate of *E. Coli* is changed depend on the amount of intracellular water [3] and cancer cells have lower number of free water molecules than normal cells [4].

The physical properties of hydration water are also different from a bulk water. In particular, the hydration water molecules are more ordered [5], diffuse more slowly [6], show slow-down in dynamic motions [7], and altered hydrogen bond structure [8]. Especially, a reduced dielectric constant of hydration water would enhance electrostatic interactions between biomolecules and stabilize its structure through strong hydrogen bonding [9]. Therefore, the water properties in the crowded environment are a key part for a deeper understanding of the behavior of intracellular biomolecules.

While ultrafast spectroscopy such as time-resolved fluorescence [10], 2D-IR [7], NMR [11] have been used for investigating the physical properties of hydration water, only local environment nearby the probe molecules could be measured. Meanwhile, since dielectric relaxation spectroscopy are sensitive to a large dipolar molecules such as water, it can access the overall properties of hydration water in the solution by analyzing reorientation motion in the GHz-THz range. However, usually crowded biomolecular solutions have a relatively large electrical conductivity due to its own surface charge. The contribution of the electric conductivity in the dielectric constant could mask water relaxation peak in the GHz range [12], it makes peak decomposition difficult.

To avoid this problem, we choose the neutral DMPC lipid bilayers as a target biomolecule and polyethylene glycol (PEG) as a crowding agent to induce intracellular environment. The PEG-induced osmotic pressure affects not only the membrane structure and function [13,14] but also the hydration water properties because the water orientation dynamics are closely related to the structure of the membrane [15,16]. In the previous studies, however, dynamical properties of water in the crowded environment is measured at far below than full hydration condition of the membrane due to the limitation of the measurement time-scale of NMR [17]. The measurement at more realistic and fully-hydrated condition is necessary in order to understand the dynamics of water in the intracellular environment. In this presentation, we present the dynamical properties of overall water dynamics of DMPC-PEG solution at fully hydration condition from the dielectric response in the GHz-THz range.

II. RESULTS

Various concentration (0~60 wt%) of PEG solutions and DMPC solution were prepared by adding DI water. Equal volume of DMPC (Avanti Polar Lipids) and PEG (Sigma) solutions were mixed and stabilized for a few hours at 303K. In order to determine the complex dielectric constants of the solution, terahertz time-domain attenuated total reflection (ATR) spectroscopy was applied in the frequency range from 0.3 THz to 1.2 THz to avoid the contribution of water vibrational motion. All the measurements were performed on TAS7500 (Advantest) with a precise temperature control. In the frequency range from 1 GHz to 40 GHz, vector network analyzer (Anritsu Vectorstar) is used with open-ended coaxial probe (Keysight 85070E). The probe is directly dipped into the solution with a same temperature controller.

The measured complex dielectric constants were fitted using widely used double-Debye type relaxation model,

$$\varepsilon^*(\omega) = \varepsilon_\infty + \frac{S_1}{1 + i\omega\tau_1} + \frac{S_2}{1 + i\omega\tau_2}$$

with fixed relaxation time of bulk water at the measured temperature (Fig. 1a). Our model assumes that hydration water molecules cannot be detected in our THz measurement range due to the sufficient slowdown of its dynamics than that of bulk water. The calculated hydration number of the DMPC (~29)

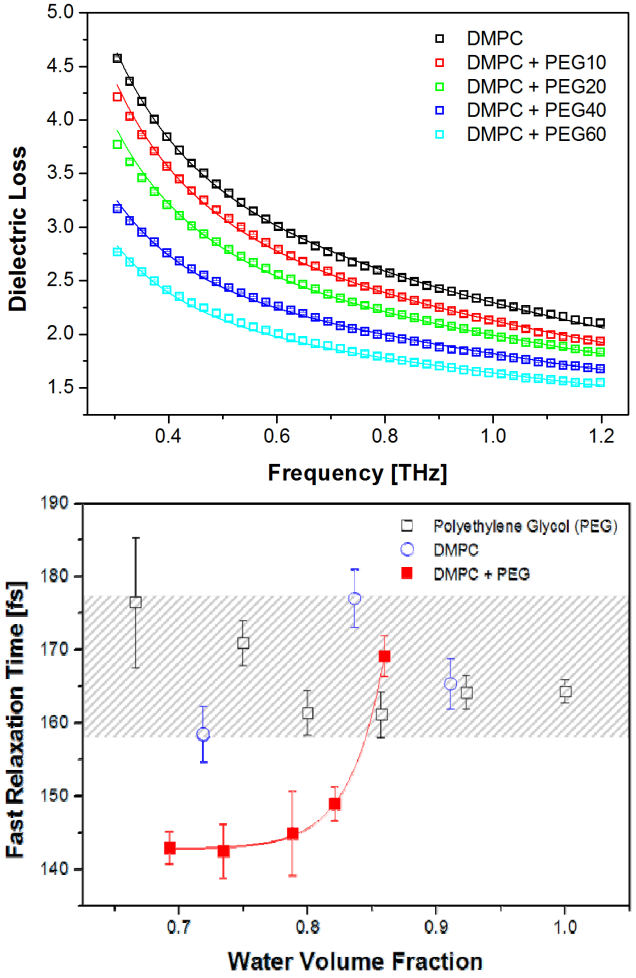

Fig. 1. (a) Dielectric loss of DMPC, DMPC-PEG solutions measured by THz-TDS (0.3-1.2 THz). The solid lines show the fitting result by using double Debye relaxation model. (b) Fast relaxation time of water molecules as a function of water volume fraction of PEG (open square), DMPC (open circle), and mixed (solid square) solution.

[15] and the EG monomer (~3) [19] are in good agreement with previous data. The DMPC or PEG solution shows constant fast water relaxation time and water destructuring with increasing the solute concentration. However, surprisingly, the relaxation time of non-hydrogen bonding (NHB) or fast component of water molecules shows accelerated behavior in the DMPC-PEG mixed solution only (Fig. 1b). The acceleration behavior may rise from the chemical potential change due to the osmotic interaction between DMPC and PEG molecules. This finding suggests that osmotic interaction may induce the structure breaking of hydrogen bonding like the confined water [8].

Actually, however, polymeric solution shows broaden water relaxation peak and need to be modeled by Cole-Cole relaxation model due to the local fluctuation of polymer chain [18] or we can add additional Debye type relaxation term [19]. In order to figure out the proper dielectric model, vector network analyzer with open-ended coaxial probe is used to obtain dielectric constant in the frequency range of 1-40 GHz. With the measurement in the GHz range, we re-analyzed the dynamic properties of hydration water even it may have little contribution in the THz range. The details will be presented at the conference.

III. Conclusion

Using polyethylene glycol, we induce model intracellular environment on neutral DMPC lipid bilayers solution. And we observed the accelerated movement of non-hydrogen bonding water molecules interacting with DMPC in polyethylene glycol solution from the dielectric response in the terahertz range by using double-Debye relaxation model. Combined with the dielectric measurement in the GHz range, detailed behavior of hydration water will be discussed at the conference.

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