Evaluation of the Bulk Water dynamics inside HeLa Cells using Terahertz Time-Domain Attenuated Total Reflection Spectroscopy

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Abstract

In spite of intensive efforts to elucidate the cellular functions and activities of biological macromolecules, such as DNA and proteins, the intermediary role of intracellular water in these activities is still cloaked in mystery. This is because bulk water dynamics, which occur on sub-picosecond and picosecond timescales, cannot be directly probed by conventional experimental techniques. With a periodic electric field on a sub-picosecond or picosecond timescale, terahertz waves are selectively sensitive to the dynamics of the hydrogen bond network associated with the bulk water. We use THz TD-ATR measurements, combined with a two-interface model, to determine the complex dielectric constant of a living cell monolayer, and thus characterize their intracellular water dynamics. Further interrogation was undertaken by decomposing the complex dielectric constant into its slow relaxation, fast relaxation and intermolecular stretching vibration components. The results indicated that intracellular water has a fragile hydrogen bond network, releasing water molecules from the network. Additionally, tetrahedrally coordinated water in the cell interior is structurally disordered compared to the extracellular water.

1. Introduction

Water actively supports biological reactions via direct or indirect interactions in various ways in living systems. Therefore, it is believed that the cellular activity (or freshness) [1] and malignancy [2-4] are closely related with intracellular water. Such peculiarities of intracellular water arise from their complex ability to interact with neighboring molecules, i.e. through their hydrogen bonds [5,6]. This interaction between the surrounding water molecules and hydrophilic biological molecules is commonly called hydration. These hydrogen bonds have a sub-nanosecond lifetime. On the other hand, bulk water, which by definition is unperturbed by any solute molecules, forms transient hydrogen bonds with much faster lifetimes of sub-picoseconds or picoseconds. In this hydrogen bond network each water molecule is capable of forming four hydrogen bonds with their neighbors in a tetrahedral formation [5], as shown in Figure 1(a). Since the hydrogen bonds between water molecules are not static, but are dynamically reconfiguring on a sub-picosecond and picosecond timescales, the structure of the hydrogen bond network of bulk water fluctuates at picoseconds. Though these peculiar water dynamics appear to be related to cellular functions and activities, only cursory discussions about intracellular water dynamics have been undertaken, except for piecemeal investigations on the hydration states of monocellular organisms [7,8]. Therefore, the roles of intracellular water are still veiled; this is because sub-picosecond and picosecond dynamics cannot be directly explored by conventional experimental techniques.

Electromagnetic waves in the terahertz (THz) region have frequencies equivalent to those bulk water and thus can be used to probe these dynamics since the oscillation period of THz waves are typically sub-picosecond and picosecond timescales and thus can be coupled with bulk water motions. Accordingly, the complex dielectric constant, which can be measured by THz spectroscopy is a quantitative reflection of the molecular reorientations and vibrations of bulk water [9,10]. Additionally, dynamical motions of biological macromolecules, such as nucleic acids and proteins are significantly slower than the dynamics associated with bulk water [11]. This means the complex dielectric constant is also selectively sensitive to bulk water dynamics alone, while any contribution from biological macromolecules and the surrounding retarded hydrated water molecules are negligibly small [12].

In this study, bulk water dynamics associated with the hydrogen bond network inside human living cells (HeLa) are investigated via complex dielectric constant measurements in the THz region. To determine the complex dielectric constant of a HeLa cell monolayer, a two-interface model was developed. In light of the experimental results, intracellular hydration state, stability of hydrogen bonds and the structure of the tetrahedral hydrogen bond network are discussed.

2. Principle

Terahertz time-domain attenuated total reflection (THz TD-ATR) spectroscopy is a promising tool to determine the complex dielectric constant of absorptive samples, such as water in the THz frequencies [13]. In this geometry,

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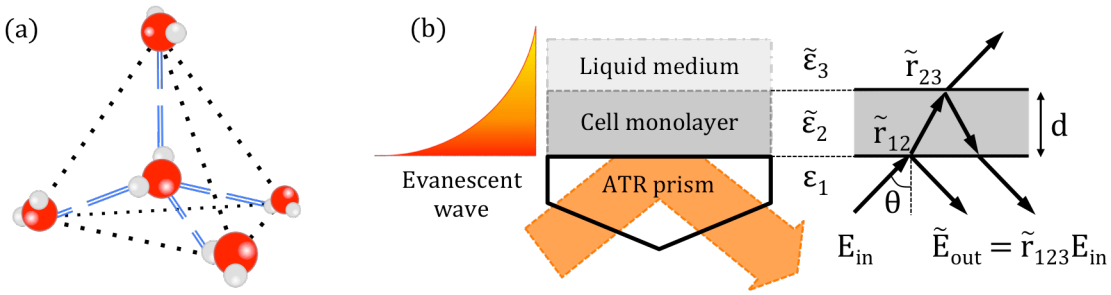

Figure 1. (a) Terhedrally coodinated hydrogen bond network of bulk water. (b) Schematic illustration of THz TD-ATR spectroscopy with a two-interface model.

when the incident temporal THz pulse with a picosecond width is totally reflected at the ATR prism – sample interface, an evanescent field penetrates into the sample with a maximum depth of tens of micrometers and interacts with the sample. As a result, by comparing the amplitude decrease and time delay between incident and reflected THz pulse, the real part and imaginary part of the complex dielectric content of the sample can be simultaneously determined. If the measurement sample is a layer thinner than the depth of the evanescent field, dielectric responses above the layer sample will also be included in the result. To selectively extract the complex dielectric constant of the sample layer alone, a two-interface model is used (see Figure 1 (b)). In this case, the "apparent" reflection coefficient $\tilde{r}_{123}$ reflects both the sample layer and bulk sample above the sample layer, but $\tilde{r}_{123}$ can be related to each reflection component using equation (1);

$$\tilde{r}_{123} = \frac{\tilde{r}_{12} + \tilde{r}_{23}\exp\left[-\frac{4\pi d\sqrt{\varepsilon_1 \sin^2\theta - \tilde{\varepsilon}_2}}{\lambda}\right]}{1 + \tilde{r}_{12}\tilde{r}_{23}\exp\left[-\frac{4\pi d\sqrt{\varepsilon_1 \sin^2\theta - \tilde{\varepsilon}_2}}{\lambda}\right]} \tag{1}$$

where, thickness of the layer sample (d), incident angle (θ), wavelength (λ), dielectric constant of the ATR prism (ε_1) and complex dielectric constant of the layer sample ($\tilde{\varepsilon}_2$) [14]. The reflection coefficient of the ATR prism –sample layer ($\tilde{r}_{12}$) and the sample layer – bulk sample ($\tilde{r}_{23}$) is expressed as;

$$\tilde{r}_{12} = \frac{\sqrt{\tilde{\varepsilon}_2}\cos\theta - \sqrt{\varepsilon_1}\sqrt{1 - \varepsilon_1/\tilde{\varepsilon}_2 \sin^2\theta}}{\sqrt{\tilde{\varepsilon}_2}\cos\theta + \sqrt{\varepsilon_1}\sqrt{1 - \varepsilon_1/\tilde{\varepsilon}_2 \sin^2\theta}} \quad , \quad \tilde{r}_{23} = \frac{\sqrt{\tilde{\varepsilon}_3}\sqrt{1 - \tilde{\varepsilon}_2/\tilde{\varepsilon}_3 \sin^2\theta} - \sqrt{\tilde{\varepsilon}_2}\sqrt{1 - \varepsilon_1/\tilde{\varepsilon}_3 \sin^2\theta}}{\sqrt{\tilde{\varepsilon}_3}\sqrt{1 - \tilde{\varepsilon}_2/\tilde{\varepsilon}_3 \sin^2\theta} + \sqrt{\tilde{\varepsilon}_2}\sqrt{1 - \varepsilon_1/\tilde{\varepsilon}_3 \sin^2\theta}} \tag{2}$$

where $\tilde{\varepsilon}_3$ represents the complex dielectric constant of the bulk sample. Simultaneous equations of (1) and (2) give the complex dielectric constant of the sample layer $\tilde{\varepsilon}_2$ removing the excess contribution of the bulk sample ($\tilde{\varepsilon}_3$), if the rest parameters are known. For further details refer to Ref. [15,16].

3. Material and Methods

We constructed a cell culture incubation chamber on the ATR prism of the spectrometer, TAS 7500 (ADVANTEST Co.). The incubation chamber was kept at 310 K with 5 % CO_2. Human cervical cancer cells, HeLa, were cultured in D-MEM/Ham's F-12 culture medium on the ATR prism [15,16]. After HeLa cells were confirmed to form a cell monolayer on the ATR prism through a digital microscope (Keyence Co., VHX-1000) set above the incubation chamber, THz TD-ATR measurements were performed. To apply the two-interface model calculation, the complex dielectric constant of the culture medium $\tilde{\varepsilon}_3$ was measured in advance and the thickness of the cell monolayer d = 7.0±1.0 μm was estimated by 3D cross-section images constructed from a scanning confocal laser microscope (Nikon, A-1).

4. Results and Discussions

The experimentally determined complex dielectric constant of the HeLa cell monolayer is compared with that of distilled water in Figure 2. The real part is almost unchanged over the whole frequency region, while on the other hand, the imaginary part shows a significant decrease for HeLa cells. To systematically understand these phenomena, the complex dielectric constant of water was decomposed into its constituents; the slow relaxation $\tilde{\chi}_{\mathrm{slow}}(\omega)$, fast relaxation $\tilde{\chi}_{\mathrm{fast}}(\omega)$, intermolecular stretching vibration $\tilde{\chi}_{\mathrm{S}}(\omega)$ and higher frequency limit ε_∞.

$$\tilde{\varepsilon}(\omega) = \tilde{\chi}_{\mathrm{slow}}(\omega) + \tilde{\chi}_{\mathrm{fast}}(\omega) + \tilde{\chi}_{\mathrm{S}}(\omega) + \varepsilon_\infty \tag{3}$$

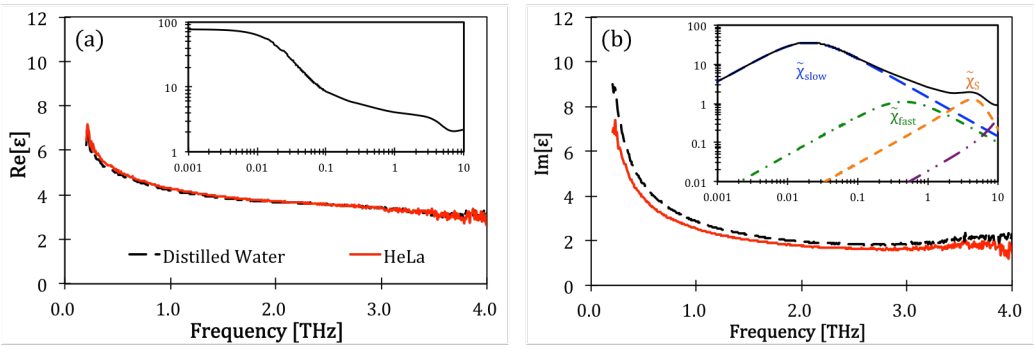

Figure 2. (a) Real part and (b) imaginary part of the complex dielectric constant. The inset shows the broadband complex dielectric constant of water from 1 GHz to 10 THz and its constituents.

$$= \frac{\Delta\varepsilon_{\text{slow}}}{1 + i\omega\tau_{\text{slow}}} + \frac{\Delta\varepsilon_{\text{fast}}}{1 + i\omega\tau_{\text{fast}}} + \frac{\Delta V_S {\omega_S}^2}{(\omega_S - \omega)^2 + i\omega\gamma_S} + \varepsilon_\infty$$

where $\Delta\varepsilon_{\text{slow(fast)}}$ and $\tau_{\text{slow(fast)}}$ are the relaxation strength and relaxation time of slow (fast) relaxation. ΔV_S, ω_S and γ_S are the vibration strength, resonant frequency and damping constant of the intermolecular stretching vibration. Since biological macromolecules largely lack picosecond dynamics in an aqueous environment, the complex dielectric constant of HeLa can be considered to be dominated by the slow relaxation, fast relaxation and intermolecular stretching vibration mode of water.

The slow relaxation mode ($\tau_{\text{fast}} \fallingdotseq 6.3$ ps) is a collective reorientation of hydrogen-bonded water molecules, whereas the fast relaxation ($\tau_{\text{slow}} \fallingdotseq 0.3$ ps) is assigned to rotational motion of a non-hydrogen-bonded individual water molecule [9]. Taking into account that the relaxation strength is proportional to the number of water molecules, the fraction of the fast relaxation strength ($\Delta\varepsilon_{\text{fast}}$) to the total relaxation strength ($\Delta\varepsilon_{\text{slow}} + \Delta\varepsilon_{\text{fast}}$) is equivalent to the fraction of water molecules isolated from (not bound by) the hydrogen bond network $f_{\text{ind.}}$ [9].

$$f_{\text{ind.}} = \frac{\Delta\varepsilon_{\text{fast}}}{\Delta\varepsilon_{\text{slow}} + \Delta\varepsilon_{\text{fast}}} \tag{4}$$

For distilled water at 310 K, $f_{\text{ind.}}$ is estimated to be 3.1 %, and in HeLa 4.7 %, which indicates the intracellular hydrogen bond network of the bulk water is more fragile (not as tightly bound) than "common" water. This result may be because of K^+, which is known to be a "structure breaker" of the hydrogen bonds network, is kept at high concentration in the cell interior and the freedom degree of the hydrogen bond network becomes smaller due to abundant intramolecular biological macromolecules.

The intermolecular stretching vibration mode is sensitive to the tetrahedral coordinated hydrogen bond network of water, and therefore the oscillation strength f_S of this mode quantitatively represents the degree of "tetrahedrality" [10].

$$f_S \propto \int \omega \text{Im}[\tilde{\chi}_S(\omega)] d\omega \tag{5}$$

As a result, the oscillation strength of distilled water (f_S^{Water}) and HeLa (f_S^{HeLa}) is found to be $f_S^{\text{Water}} : f_S^{\text{HeLa}} = 1 : 0.5$. This attenuation in f_S^{HeLa} partly originates from the decreased mol concentration of bulk water inside living cells, i.e. some bulk water molecules are replaced by solutes, while others are withdrawn into the hydrated water. Considering the decreased concentration of bulk water in HeLa, in which solute molecules and hydrated water occupy 16 % [17] and 22 % (calculated from our work but not shown here) respectively, only 62 % in HeLa is assumed to be composed of bulk water. In this assumption, $0.62 \times f_S^{\text{Water}}$ is still larger than f_S^{HeLa}, which suggests intracellular bulk water is partially constrained from contributing to intermolecular stretching vibration. Therefore, we suggest the hydrogen bond network of bulk water in HeLa cells cannot be considered to be in a true tetrahedral formation.

6. Conclusion

The complex dielectric constant of HeLa living cells was determined by THz TD-ATR measurement combined with a two-interface model. Since the complex dielectric constant in the THz region is selectively sensitive to sub-picosecond and picosecond bulk water dynamics, bulk water dynamics can be decomposed into slow relaxation, fast relaxation and intermolecular stretching vibration components. As a result, we found intracellular bulk water forms a

fragile hydrogen bond network, with a much less tightly bound tetrahedral structure than seen in extracellular bulk water. Our results indicate THz spectroscopy can characterize intracellular water dynamics that previously couldn't be experimentally and computationally achieved, and therefore opens up a new avenue for evaluating cellular functions and activities in terms of "intracellular water dynamics."

7. References

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