Effects of Hydration on Protein Dynamics and its Thermal Excitation Studied by Broadband Dielectric Spectroscopy

Naoki Yamamoto[1], Shota Ito[2], Hideki Kandori[2], Kaoru Ohta[3], Atsuo Tamura[1], Eri Chatani[1], Keisuke Tominaga[1,3]

[1]*Graduate School of Science, Kobe University*

[2]*Graduate School of Engineering, Nagoya Institute of Technology*

[3]*Molecular Photoscience Research Center, Kobe University*

Summary: Broadband dielectric spectra were obtained on several globular and membrane proteins from 0.5 gigahertz (GHz) to 1.8 terahertz (THz) at room temperature as well as their temperature dependence at 0.3-1.8 THz region. In the case of a globular protein, lysozyme, we found that there was a large relaxational mode in the GHz region, which was assigned as a protein-hydration water-coupled relaxational mode. Furthermore, a high-frequency tail of the relaxational mode heavily overlapped with the THz region. Based on a spectral analysis of the temperature-dependent complex dielectric spectra in the THz region, we found that the relaxational mode started to overlap with the THz region from 200 K whereas vibrational modes intrinsic in the THz region mildly contributed to the increase in the spectral components from 180 K. In the case of purple membrane, we found a essentially similar result to that of the globular proteins.

Introduction: Protein function is exhibited in the thermal fluctuation of the solvent, water. Therefore understanding the dynamics of the hydrated protein and its thermal excitation is crucial for elucidating the relationship between the protein dynamics and the function. It has been known that water fluctuation is characterized by the time scale of nanosecond and picosecond, which correspond to the frequencies of the electromagnetic wave of GHz and THz. In this study we measured temperature and hydration dependence of the complex dielectric spectra in the frequency regions on globular and membrane proteins.

Methods: Several globular proteins such as lysozyme were purchased and purple membrane was expressed and purified by a well-known procedure. Their hydration levels were appropriately controlled by varying water vapor pressure. The complex dielectric spectra were obtained using a vector network analyzer (0.5-20 GHz) and two THz time-domain spectrometers (0.1-0.3 THz or 0.3-1.8 THz, respectively).

Results and Discussion: Figure 1 shows the complex dielectric spectra of purple membrane at room temperature (293 K) at h = 0.30 where h indicates weight of water divided by weight of protein. Referring to a report on lysozyme [1], we performed a spectral fitting using the equation below:

$$\varepsilon^*(\nu) = \frac{\sigma_0}{i2\pi\nu\varepsilon_0} + \frac{\Delta\varepsilon_j}{1+(i2\pi\nu\tau)^{\beta_j}} + \sum_{k=1}^{2}\frac{A_k}{\nu_k^2 - \nu^2 + i\nu\gamma_k} + \varepsilon_{\inf}.$$

The first term represents the contribution of an ionic conductivity in the low-frequency region of the imaginary part. The 2nd term is two cole-cole functions representing a relaxational mode in the GHz region, and the third term indicates two underdamped modes for two vibrational modes in the THz region. As shown in figure 1, the relaxational mode is the a main component in the GHz region overlapping with the THz region. Based on the result, temperature dependence of the complex dielectric spectra in the THz region was analyzed. As a result we found that the overlap in THz region started from 220 K while a much fewer contribution of the vibrational modes was confirmed from 180 K. The result provides a suggestion for the interpretation of a "protein dynamical transition" that has been debated in neutron scattering experiments [2].

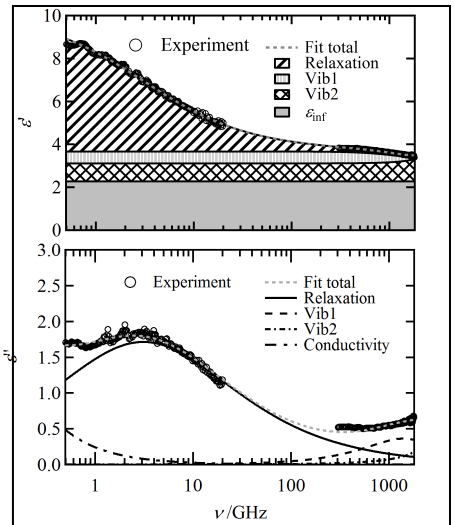

Figure 1. Broadband complex dielectric spectra of hydrated purple membrane at 293 K.

References:

1. N. Yamamoto, K. Ohta, A. Tamura, and K. Tominaga, *J. Phys. Chem. B*, **120(21)**, 4743-4755, 2016.

2. S. Magazù, F. Migliardo, A. Benedetto, *J. Phys. Chem. B*, **115**, 7736-7743, 2011.