Terahertz Spectroscopy of Protein in Water

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***Abstract*— We have performed a terahertz time-domain spectroscopy for a protein in a reverse micelle. Absorption of terahertz wave due to solvent is greatly reduced in this sample, compared with in protein in aqueous solution. Absorption spectra due to the protein molecule have been derived.**

I. INTRODUCTION AND BACKGROUND

IT is believed that low-frequency vibrational motions of proteins play an important role on their functions. THz spectroscopy[1] as well as inelastic neutron scattering[2] measurements is a suitable method to investigate such vibrational motions. Most of the studies using such experimental methods have been done for lyophilized proteins with a certain hydration level, because background signal due to solvent waters is huge compared with signal due to protein molecules in protein aqueous solutions. It is important to investigate low-frequency vibrations of a protein in water because proteins work in an aqueous solution. A protein in a reversed micelle is a candidate for THz spectroscopy of protein in aqueous solution. As seen in Fig.1, the protein aqueous solution is confined in the nanometer-sized reversed micelle[3], while the solvent is oil (nonpolar liquid) whose THz absorption is much weaker than that due to solvent waters. Therefore, the water content of the sample is very little.

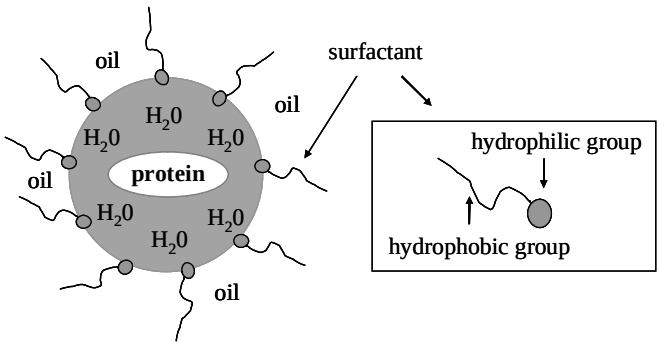

Fig.1. Schemitic of a protein in a reverse micelle.

The size of a reverse micelle is variable by changing a water-surfactant molar ratio, and affects hydrogen-bond network of water in a reverse micelle[4]. That is, the bulk-like water content of a reverse micelle is low in a small water-surfactant molar ratio. Therefore, THz spectroscopy of a protein in a reverse micelle as a function of the molar ratio is expected to become a method to study the effect of the surrounding waters on dynamical behavior of a protein.

On the other hand, most of the studies using such experimental methods focus on systems in the thermal equilibrium state. However, in order to clarify the effect of the low-frequency motion on biomolecular functions, it is important to investigate the low-frequency motion during the functional process. The investigation is considered to be possible by pump-probe THz spectroscopy. That is, low-frequency motions of a biomolecule are probed by the THz field pulse at an arbitrary time after its function is initiated by a laser pulse. For this end, we have developed a measurement system for an optical-pump THz-probe spectroscopy, in which an electro-optic (EO) sampling of THz field is achieved by a combination of a chirped pulse and multi-channel spectrometer[5]. In the pump-probe measurement, it is required to use protein aqueous solution as a sample because a protein works under physiological conditions.

Thus, in the present study, we have performed THz spectroscopy of a protein in a reverse micelle in order to verify that signal due to the protein is obtained from the measurement. Moreover, the dependence of a water-surfactant molar ratio on the spectrum has been measured.

II. EXPERIMENTAL

A surfactant, sodium bis(2-ethylhexyl) sulfosuccinate (AOT) was dissolved in a non-polar solvent, isooctane. Myoglobin in milipore water was injected into the AOT/isooctane solution. The sample was condensed through evaporation of isooctane in a vacuum desiccator. The concentration of myoglobin in the sample was varied from 10^{-4} to 10^{-3} mol/L. A Karl Fisher titrator was used to measure the water content of the sample.

The THz time-domain spectroscopy[1] was done to obtain absorption spectra of the sample using a 150-fs Ti:sapphire laser with a 1-kHz regenerative amplifier up to a pulse energy of several hundred μJ (Fig.2). The laser pulse was divided into two beams. One was used to generate a THz field pulse

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by irradiating it on an InAs substrate, and the other was used for EO sampling of THz field waveform with a 1-mm ZnTe crystal after passing through an optical delay line. A 10-mm thick cell with two sample rooms (double cell) was used: one is for isooctane and the other is for the protein solution. The temporal waveform of THz field pulse transmitted through the two samples was measured by turns by moving the cell. The double cell reduces the time interval between the two measurements, leading to a small effect of a long-period fluctuation of the laser on derivation of the absorption spectrum from the two data. The measurement system was in a nitrogen purge box to eliminate the absorption of the THz wave due to water vapor.

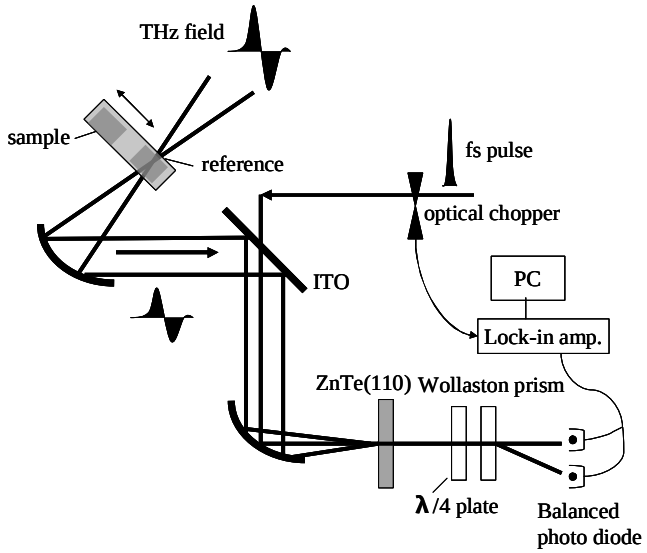

Fig.2. Experimental setup of THz-TDS with a double cell.

III. RESULTS

Figure 3 shows the absorption spectra of the sample with two concentrations of myoglobin, where the concentrations of AOT and water are 0.5 and 0.1 mol/L, respectively. The amplitude of the absorption spectrum increases with increasing the concentration of myoglobin. Since only the concentration of the protein varies, the difference between the two spectra is attributed to the protein molecule. In the presentation, we will discuss the results in detail, together with the result of measurement as a function of the water-surfactant molar ratio.

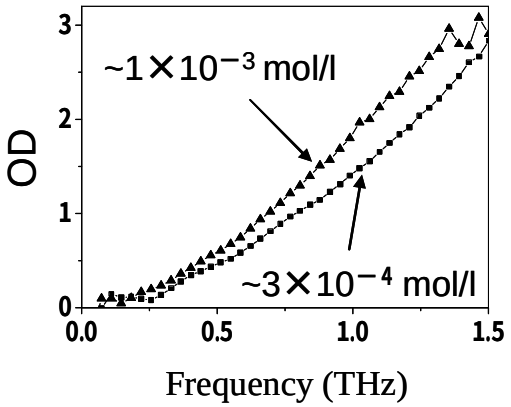

Fig.3. The absorption spectrum of myoglobin in AOT/isooctane reverse micelle at room temperature.

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