Broadband Dielectric Spectroscopy of Albumin-Water Mixture in Frozen State

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Protein is one of the most important biomolecules and a lot of research works for various kinds of proteins have been carried out. Particularly, various phenomena have been observed for protein-water mixtures in the frozen state. For example, the tuna, which contains much of protein, shows a glass transition determined by differential scanning calorimetry (DSC) in a temperature range of -60 - -70°C in the its frozen state. The glass transition was observed also for the aqueous solutions of globular protein at the same temperature range. Although various studies on the glass transition in]the frozen state have been carried out by thermal measurements, molecular mechanism of the glass transition has not been made clear enough yet because of lack of the information from molecular dynamics. Since glass transition originates from the molecular dynamics, we performed broadband dielectric measurements to study the glass transition. The purpose of the present work is to explain the glass transition observed by DSC measurements from the molecular dynamics in the frozen state.

The bovine serum albumin (BSA) used in this experiment was purchased from SIGMA. 40wt% albumin -water mixture was prepared. The temperature range for dielectric measurements on albumin-water mixture was between -100°C and 25°C including a melting and the glass transition temperatures. Dielectric measurements were performed in the frequency range from 2 mHz to 20 GHz, using a time domain reflectometry (TDR) (100 MHz–20 GHz), an impedance/material analyzer (Hewlett Packard 4291A) (1MHz–1.8 GHz), a precision impedance analyzer (Agilent Technologies 4294A)(40 Hz-110 MHz), an LCR meter (Hewlett Packard 4284A)(20 Hz–1 MHz), and an AC phase analysis (ACPA) method (2 mHz-1 kHz) .

Figure 1 shows dielectric constant and loss for the albumin-water mixture at -60°C by way of example as the albumin-water mixture under the frozen state. Contributions of an electrode polarization and dc conductivity were observed in the lower frequency side in the frequency range. A relaxation process for ice is shown in a frequency range of 100Hz - 10kHz. The relaxation process due to unfreezable water, remaining in the liquid state even below the melting temperature of the albumin-water mixture, was observed in the frequency range higher than 1MHz. Another relaxation process, δ-relaxation, as shown in Fig.1, is also observed in addition to these relaxation processes. The loss peak frequency has a large temperature dependence. This process is observed below 1Hz at the glass transition temperature, and shifts to higher frequency with increasing temperature up to MHz range at just below the melting temperature. This relaxation process must be concerned with the glass transition observed by thermal analysis under the frozen state. We will present more detailed discussions for these relaxation processes.

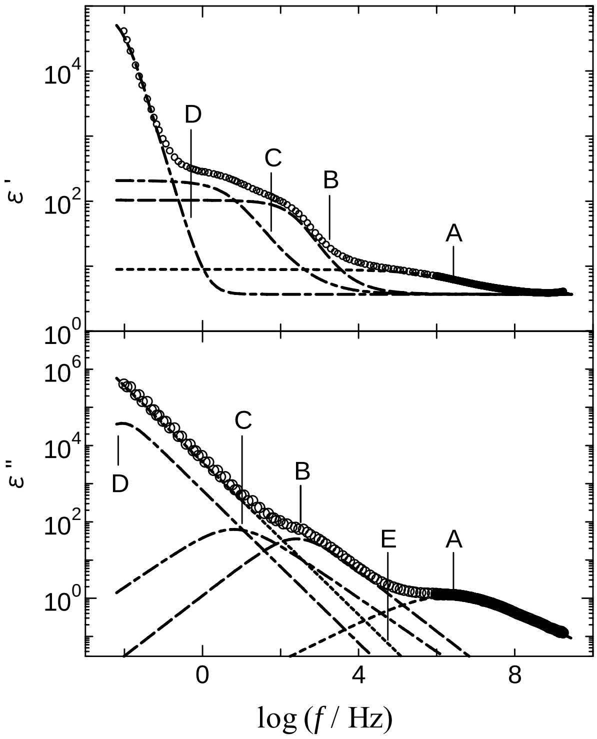

Fig.1 Dielectric constant and loss for 40wt% BSA-water mixture at -60°C. The lines drawn in the figure indicate respective processes are A; unfreezable water, B; ice, C; δ-relaxation, D; electrode polarization, and E; dc conductivity.