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Elastic propaties of orthorhombic Lysozyme crystals in cryoprotective solutions

低温凍結保護物質中におけるリゾチーム斜方晶系単結晶の弾性的性質

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1. Introduction

Protein is indispensable for life. Natural, non-native protein expression regardless of the function takes a three-dimensional structure. The physical properties of proteins have been extensively studied on various techniques[1]. It is important to understand the properties of the crystalline states with the regular inter-molecular position rather than a random positional relationship among molecules. A protein crystal is a periodic arrangement of molecules as three-dimensional crystals as same as other organic substances. Considering a large size of a protein molecule, it is necessary to form hydrogen bonds between protein and water molecules in a crystal. Moreover, such hydration induces large fluctuations, and it also affects the physical properties of a crystal[2,3]. Generally, the protein activity is inhibited in low temperatures. However, it can prevent cell damage or freezing at low temperatures by using cryoprotectants. In order to deepen the knowledge about the dynamical physical properties of proteins, we investigate the temperature dependence of elastic properties of orthorhombic HEWL (Hen egg white lysozyme) crystals by using Micro-Brillouin scattering spectroscopy[4,5].

2. Experimental

Micro-Brillouin scattering instrument used for measuring gigahertz range dynamics. The exciting source of Brillouin scattering was a green YAG laser with λ=532 nm wavelength. The Brillouin scattering spectra were measured at the backward scattering geometry. The Sandercock-type 3 + 3 passes tandem Fabry-Perot Interferomter(FPI) was combined with an optical microscope and operated to acquire the spectra of scattering light. A free spectral range of 30 GHz was applied and the Brillouin spectra were measured between ±25 GHz frequency range. The sample temperature was controlled by a cryostat cell (LINKAM THMS 600, TMS94, LNP94/2). The stability of the sample temperature was within ±0.1 °C in whole experiment. Heating and cooling rates were 5 °C/min.

HEWL orthorhombic crystal is grown by the two liquid interface method[6]. The two-liquid interface method uses an insoluble dense liquid such as fluorinate. Crystals were grown on interface of two liquids of lysozyme solution and dense liquid. The HEWL orthorhombic crystals were grown using 55 mg/ml lysozyme and 3 % (w/v) NaCl in 50 mM acetate buffer solution (pH = 4.5)at 38 °C . All crystals had developed in few days. The as grown orthorhombic crystal was shown in **Fig. 1.**

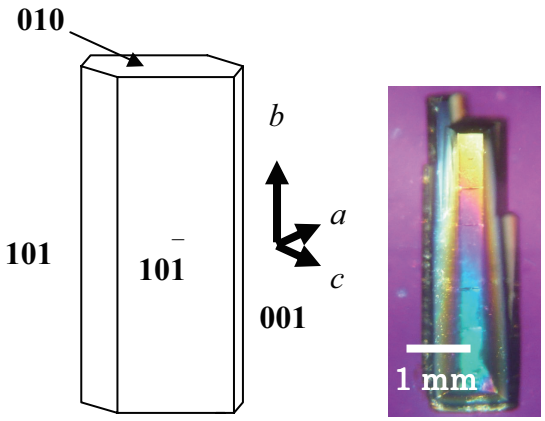

Fig.1 Micrograph of an orthorhombic HEWL crystal and its crystallographic orientation.

3. Results and Discussion

To examine the effect of cryoprotective solutions , orthorhombic HEWL protein crystals are soaked in some cryoprotective solutions (Glycerol 100mol%, glycerol 60 mol%, propylene glycol 100mol%, propylene glycil 50 mol%, dimethyl sulfoxide 30mol%). The observed Brillouin spectra of HEWL crystal and solution at three temperatures are shown in **Fig. 2**. It shows Briillouin peaks from the longitudinal acoustic (LA) mode of an orthorhombic HEWL crystal and solution. There are two doublets of peaks, inner peaks (-45 ° C about -19 GHz Stokes peaks) are LA mode peaks of

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solutions. Outer peaks (-45 ° C about -21 GHz Stokes peaks) are LA mode peaks of an orthorhombic HEWL crystal. Brillouin shift decrease and FWHM (Full Width Half at Maximum) gradually becomes broad when the temperature increases as shown in Fig. 2.

The Brillouin shift $\upsilon(=qV_L/2\pi)$ and FWHM Γ were determined by fitting from the spectra. The longitudinal sound velocity V_L was determined from Brillouin shift and scattering wave vector q $[=2\pi n\sin(\theta/2)/\lambda]$ of the backward scattering geometrty, where n, λ, and θ are the refractive index of a sample, the wavelength of a laser, and scattering angle, respectively. The LA mode attenuation coefficient of α is related to FWHM of the Brillouin component by the equation, $\alpha=\pi\Gamma/V_L$.

The temperature dependences of sound velocity and attenuation are plotted as shown in **Fig. 3.** The temperature dependence of structural relaxation time was determined from Sound velocity and absorption coefficient by the assumption of the Debye-type a single relaxation. The attempt frequency f_0, attempt relaxation time τ_0 and activation energy ΔE were determined by the Arrehenius law $\tau = \tau_0 \exp(\Delta E/RT)$. It is found that the obtained temperature dependence obeys the Arrehenius law. In addition, the log τ_0 vs. ΔE plot indicates that the Mayer-Neldel rule holds[7].

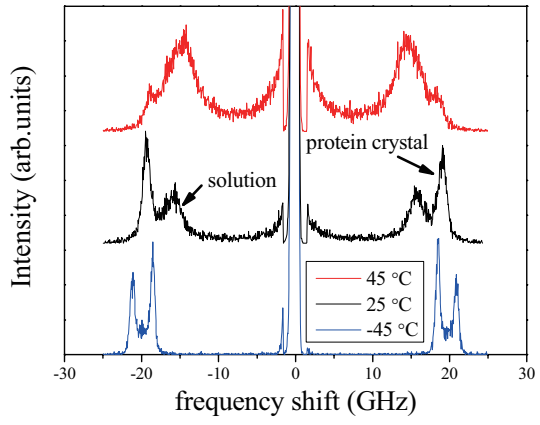

Fig. 2 Temperature dependence of Brillouin scattering spectra of an orthorhombic HEWL crystal in aqueous GL100 mol% solution. Inner and outer peaks between 10 and 22 GHz are caused by the scattering of LA modes from a crystal and a solution, respectively.

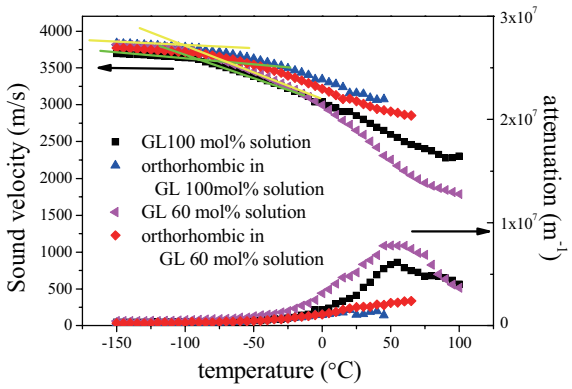

Fig. 3 Temperature dependences of sound velocity and attenuation of an orthorhombic HEWL crystal in aqueous GL 100 mol%, GL 60 mol% solutions.

4. Conclusion

A HEWL (Hen egg white lysozyme) orthorhombic crystal is grown by the two liquid interface method. The dynamics in a gigahertz range is measured over the wide temperature range in cryoprotective solutions by using micro-Brillouin scattering spectroscopy. The elastic anomaly and structural relaxation are clearly observed. The temperature dependence of the relaxation time obeys the Arrhenius law at low temperatures. The correlation is found between the activation energy ΔE and the pre-exponential factor τ_0.

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