

## Brillouin Scattering of Lysozyme Crystals in Aqueous Lower Alcohol Solutions

低級アルコール水溶液中のリゾチーム結晶のブリルアン散乱

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### 1. Introduction

The physical properties of protein crystals are very important in various fields such as solid-state physics, condensed-matter physics, biology, drug and food industry etc. Although structures of protein crystals have been well studied by X-ray and neutron diffraction method, elastic properties and relaxation phenomenon of protein crystal are still unclear.

The cryopreservation of the biological substances attracted much attention in biotechnology and food science. Usually the water molecules in a cell are crystallized when the biological substance is cooled and it freezes. Thus the cell is destroyed, and to make matters worse, the higher-order structure is changed. Therefore the biological substances are immersed in cryoprotectants to prevent this problem. Cryoprotectants are Lower alcohol (Propylene glycol (PG), Ethylene glycol (EG), Glycerol, etc.) have high vitrifying tendency.

In the present study, we measure Brillouin scattering of a protein crystal immersed in cryoprotectants to investigate the interaction between protein crystal and cryoprotectants.

### 2. Experimental

Tetragonal hen egg white Lysozyme (HEWL) crystals were grown by two liquids interface (TLI) method which employs insoluble and dense liquids.<sup>1</sup> The crystals were grown and float on the interface of two different kind of liquids (HEWL mother solution and dense liquid of Fluorinert) at 298 K. HEWL mother solution was consisted of 25 mg/mL HEWL and 5 % (w/v) NaCl in 50 mM acetate buffer solution (pH = 4.5).<sup>2</sup>

The Brillouin scattering spectra were measured at the backward scattering geometry. Brillouin scattering was measured by using a diode-pumped solid-state laser at 532 nm wavelength and a Sandercock type 3+3 pass tandem

Fabry-Perot Interferometer (FPI) was combined with microscope and operated to acquire spectra of scattered light. A free spectral range of 30 GHz was applied and the Brillouin spectra were measured in  $\pm 25$  GHz frequency range. The sample temperature was controlled within  $\pm 0.1$  °C and increased from room temperature with a cryogenic cell (LINKAM HTMS600).<sup>3,4</sup>

Brillouin scattering was measured in a tetragonal HEWL crystal is immersed in aqueous lower alcohol solution. The laser beam was incident perpendicular to the {110} habit plane of the crystal.

### 3. Results and Discussion

Brillouin spectra of a tetragonal HEWL crystal in aqueous PG 50 mol% solution at 223, 273, 323 K are shown in Fig. 1. Low a frequency peaks are from aqueous solution, while high a frequency peaks are from a crystal. Fig. 1 indicates that Brillouin shift increases with decreasing temperature.

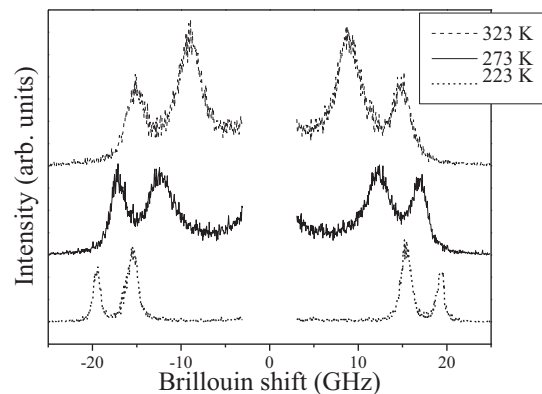


Fig. 1 Observed Brillouin spectra of a tetragonal HEWL crystal in aqueous PG 50 mol% solution.

Brillouin shift ( $\Delta\nu_B$ ) and full width at half maximum (FWHM) ( $I$ ) of Brillouin peak were determined from the spectra. We determined sound velocity ( $V$ ) and attenuation ( $\alpha$ ) from these two parameters. Sound velocity was calculated from Brillouin shift by the equation,  $V = \Delta\nu_B \lambda / 2n \sin(\theta/2)$ , where  $n$ ,  $\lambda$  and  $\theta$  are the values of refractive index of a sample, the wavelength of the laser (= 532 nm) and the scattering angle (=

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180 °), respectively. The value of refractive index of a HEWL crystal was reported in the literature.<sup>5</sup> Attenuation is related to FWHM of Brillouin component by the equation,  $a = \pi I/V$ . The temperature dependence of sound velocity and attenuation is plotted as shown in Fig. 2. About 160 K, aqueous PG 50 mol% solution with a tetragonal HEWL crystal vitrified at about 190 K, a tetragonal HEWL crystal in aqueous PG 50 mol% also. We analyzed the data of a tetragonal HEWL crystal in aqueous lower alcohol solution and calculated the relaxation time ( $\tau$ ) by the following equation.

$$\tau = \frac{\omega_m}{\omega^2} \left\{ \left( \frac{\Delta\Gamma_m}{\Delta\Gamma} \right) + \sqrt{\left( \frac{\Delta\Gamma_m}{\Delta\Gamma} \right)^2 - \left( \frac{\omega}{\omega_m} \right)^2} \right\} \quad (T < T_m)$$

The attempt frequency ( $f_0$ ), the pre-exponential factor ( $\tau_0$ ) and activation energy ( $\Delta E$ ) was determined by the Arrhenius law,  $\tau = \tau_0 \exp(\Delta E/RT)$ . We plotted the pre-exponential factor against activation energy of a tetragonal HEWL crystal in aqueous lower alcohol solution (Fig. 3).<sup>6</sup> It is found that the correlation holds between the pre-exponential factors and activation energies.

#### 4. Conclusion

By using the micro-Brillouin scattering method, the temperature dependence of a tetragonal HEWL crystal in aqueous PG solutions is measured. The glass transition of a tetragonal HEWL crystal in aqueous PG 50 mol% solution is observed. It the correlation is found between the pre-exponential factors and activation energies of a tetragonal HEWL crystal in aqueous lower alcohol solutions.

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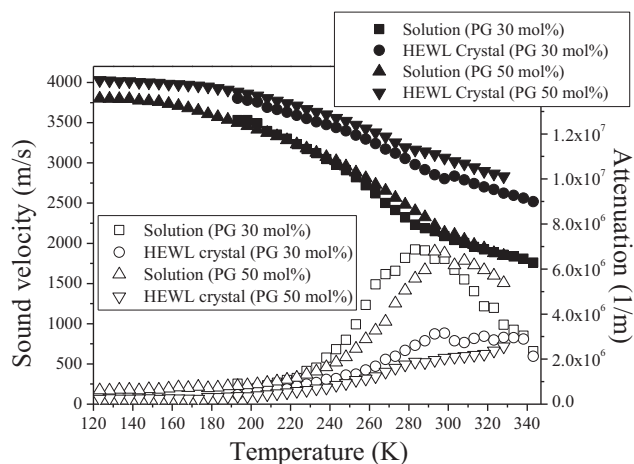


Fig. 2 Temperature dependences of sound velocity and attenuation of a tetragonal HEWL crystal in aqueous PG solution.

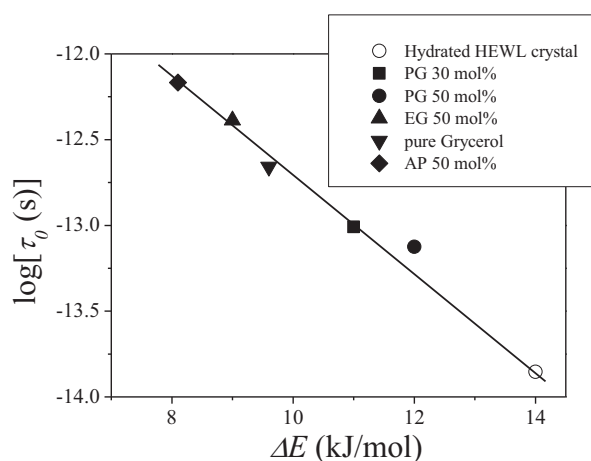


Fig. 3 The correlation between pre-exponential factor ( $\tau_0$ ) and activation energy ( $\Delta E$ ) of the Arrhenius law.

#### References

1. H. Adachi, T. Watanabe, M. Yoshimura, Y. Mori and T. Sasaki: Jpn. J. Appl. Phys. **41** (2002) L726.
2. E. Hashimoto, Y. Aoki, Y. Seshimo, K. Sasanuma, Y. Ike and S. Kojima: Jpn. J. Appl. Phys. **47** (2008) 3839.
3. Y. Ike, Y. Seshimo and S. Kojima: J. Mol. Struct. **127** (2009) 924.
4. K. Sasanuma, Y. Seshimo, E. Hashimoto, Y. Ike and S. Kojima: Jpn. J. Appl. Phys. **47** (2008) 3843.
5. B. Cervelle, F. Cesbron, J. Bethou and P. Jolles: Acta Crystallogr. Sect. A **30** (1974) 645.
6. Y. Ike, E. Hashimoto, Y. Aoki, H. Kanazawa and S. Kojima: J. Mol. Struct. **157** (2009) 924.