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Growth of Lysozyme Crystal under Static and Homogeneous Magnetic Field

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The concentration distribution of lysozyme solution was observed by using an interferometer in real-time with the different supersaturation, and the influence of a magnetic field on the growth process was discussed. In conclusion, the difference of the concentration gradient under 0T and 5T becomes large as the sapersaturation becomes high. It found that the magnetic field affects interface kinetics.
Key words: static magnetic field, microscopic interferomer, hen-egg-lysozyme, supersaturation, *in-situ* observation

1. Introduction

Quality improvement of protein crystal is important for structure determination of the crystal. It has been supposed that high quality crystal may be grown due to luck of a convection and sedimentation in microgravity [1]. Recently, we found that protein crystal growth rate of lysozyme crystal decreased under static magnetic field of 4 T compared with that under 0 T [2,3]. Therefore, the quality of crystal is expected to be improved by utilization of static magnetic field as well as microgravity, and many researchers reported protein crystal growth under a magnetic field [4-7]. However, the mechanism of the growth process under a magnetic field is not clear quantitatively.

In the present study, *in-situ* observation of the influence of a magnetic field on growth processes was conducted by using the interferometer with the different initial supersaturation.

2. Experimental

In order to observe the Lysozyme crystal growth process in real-time, the Mach-Zehnder type microscopic interferometer was placed in superconducting magnet (bore size: Ø 300 mm×600 mm, maximum magnetic induction: 6 T, inhomogeneity of the magnetic field: 1.5 % within a cylindrical space Ø 50 mm×20 mm). The experimental setups shown as Fig. 1 and Fig. 2.

A lysozyme solution (concentration of the as prepared solution: 2.0 wt% hen egg-white lysozyme HEWL: Seikagaku kogyo, six times recrystallization, 4.0 wt% NaCl, pH = 4.60) was prepared and subsequently poured to the quartz cells shown as Fig. 3. The cell was set at the optical path of the microscopic interferometer to be perpendicular to the direction of a magnetic field. The seed crystals, whose oriented were (110) regularly, was grown up at the bottom of a cell under a magnetic field. Fig. 4 is a photo of the oriented crystals under 6 T [3]. The seed crystals in the growth cell are arranged regularly in this way.

Different supersaturation in the solution were achieved by a step cooling method, and the crystal was grown up for the direction opposite to gravity (supersaturation: $\beta = C/Ce = 1.5$; growth: from 29 °C to 23 °C, $\beta = 2.3$; growth: from 29 °C to 19 °C, $\beta = 3.0$; growth: 29 °C to 16 °C (Fig. 5) [8]). Fig. 6 is the temperature profiles in the cell. *In-situ* observation of these interference fringes was carried out. Fig. 7 is the interference fringes image in crystal growth cell by microscopic interferometer under 5 T. The concentration gradient ($\partial c/\partial y$) of lysozyme solution in the solid/liquid (S/L) interface was calculated by the following equations,

$$\frac{\partial c}{\partial y} = \frac{\partial c}{\partial n} \cdot \frac{\partial n}{\partial y}, \quad (1)$$

$$\frac{\partial n}{\partial y} = \frac{\lambda}{d \cdot l} \cot\theta, \quad (2)$$

where, c: concentration of the solution, n: refractive index, λ: wavelength of incident, d: thickness of lysozyme solution, and l: distance between fringes [2,9]. Fig. 8 shows the schematic of interference fringes of crystal growth and measurement point.

3. Results and Discussion

Fig. 9 shows the concentration gradients of lysozyme solution at the different supersaturation under static magnetic field of 0 T and 5 T. Our experiment was from the high supersaturation solution ($\beta > 3.0$) and then, crystal growth and dissolution rates were degreased [2,3]. In this study, in the low supersaturation ($\beta = 1.5$), the concentration gradient under 5 T not change than that under 0 T (Fig. 9 (a)). However, we found that the concentration gradient change in the

growth from $\beta = 3.0$ and $\beta = 2.3$ solution decrease under 5 T compared with that under 0 T (Fig. 9 (b) and (c)). The magnetic field influenced strongly growth rate at the time of the high supersaturation. The influence of the magnetic field on a conducting fluid is given by the Hartmann number H_a, which represents the relation of the electromagnetic force to the viscosity,

$$H_a = B_0 d\sqrt{\sigma / \rho_0 \upsilon}, \qquad (3)$$

where B_0: static magnetic induction, $d = 1.0 \times 10^{-3}$ m, $\sigma = 13\ \Omega^{-1}\ m^{-1}$: electrical conductivity of the solution, $\rho_0 = 1.0$: density of lysozyme solution, and $\nu = 9.8 \times 10^{-7}\ m^2 s^{-1}$: kinematic viscosity [10]. At $B_0 = 5$ T, $H_a = 18.2$. Magnetic field does not show obvious effect on the convection in our experimental conditions as it does on metal or semiconductor solution growth [11,12]. Therefore, it is thought that magnetic field influences not convection but interface kinetics.

4. Conclusion

We found that the difference of the concentration gradient under 0 T and 5 T becomes large as the sapersaturation becomes higher. Thus, it is thought that the kinetics of the S/L interface is affected by the magnetic field.

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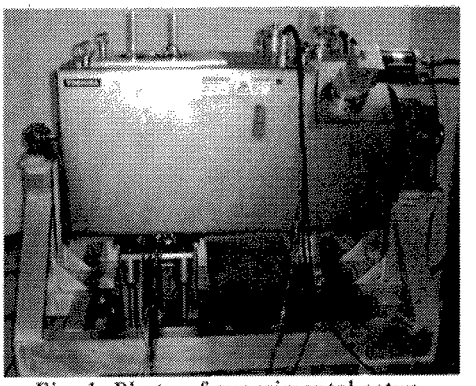


Fig. 1. Photo of experimental setup.

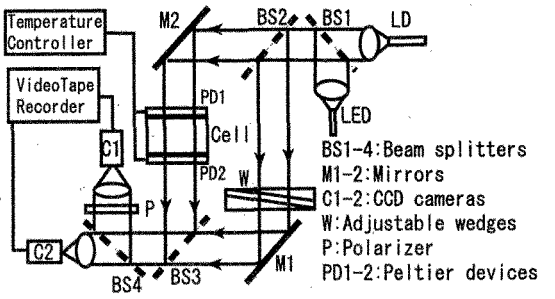


Fig. 2. Schematic drawing of microscopic interferometer.

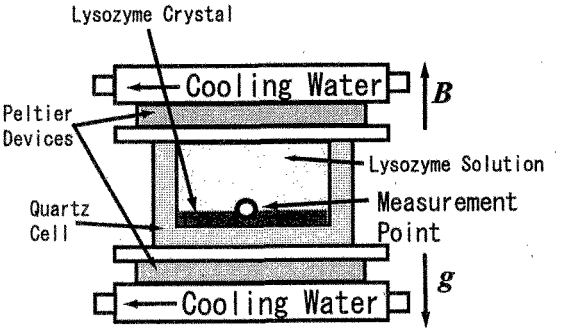


Fig. 3. Schematic of crystal growth cell for protein.

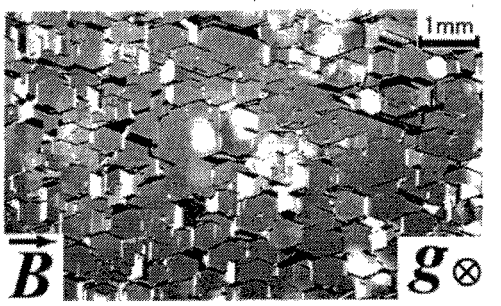


Fig. 4. Photo of lysozyme crystals grown under static magnetic field of 6T with the (110) surface [3].

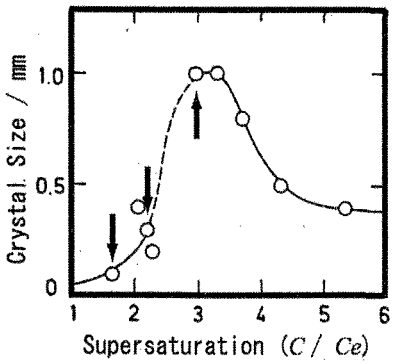


Fig. 5. Average crystal size change with degree of supersaturation (*C*/*Ce*) [8]. The arrows correspond to the present experimental conditions.

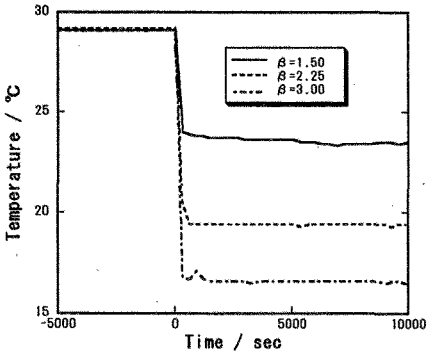


Fig. 6. Temperature profiles of crystal growth cell.

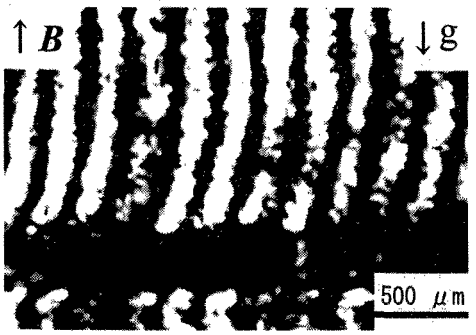


Fig. 7. Interference fringe image of crystal growth cell by microscopic interferometer under 5 T.

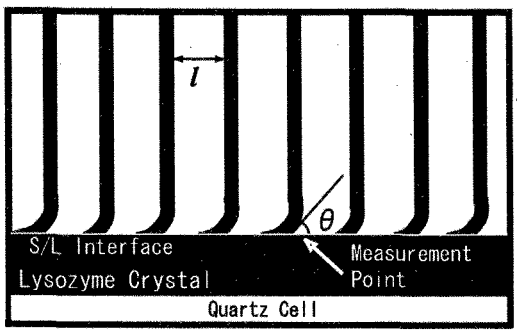
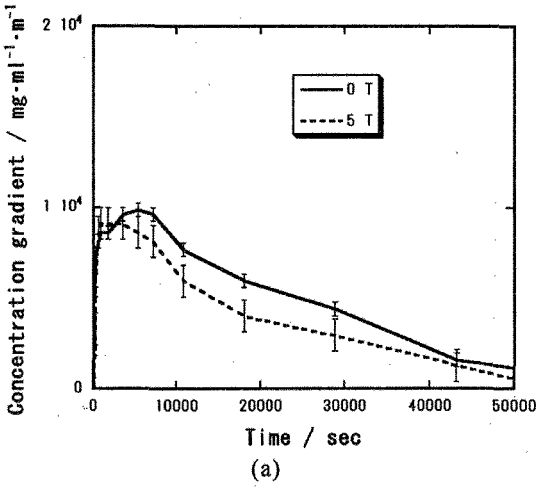
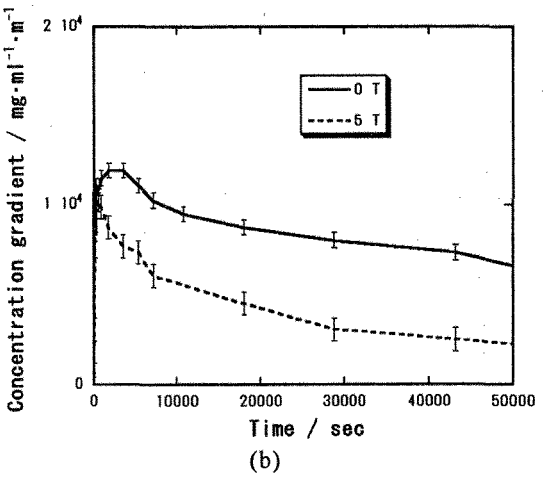


Fig. 8. Schematic of interference fringes of crystal growth.





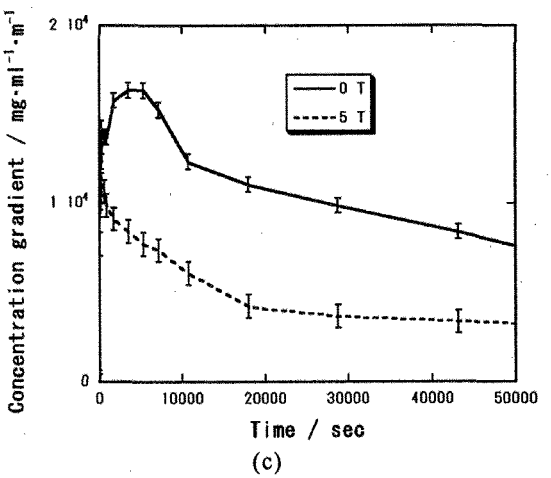


Fig. 9. Concentration gradient of lysozyme solution: (a) *C*/*Ce* = 1.50, (b) *C*/*Ce* = 2.25, (c) *C*/*Ce* = 3.00. The err-bars correspond to measurement errors of angle θ.

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