Crystallization of Lysozyme by LLIP Method under High Magnetic fields III

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The liquid-liquid interfacial precipitation (LLIP) method is one of crystallization techniques using a good and poor solvent for a precipitant. The crystal seed is borne and grown in the supersaturated layer at around an interface between them due to the counter diffusion.[1] We have reported previously the magnetic field effects on the size, morphology, and crystal structure for the crystals of C60 fullerene, Glycine, Taurine, etc.[2-4] The size of Lysozyme enlarges as a magnetic field effect under the influence of magnetic field.[5] The size of crystal is depended on the magnetic force field which is product of the magnetic field and its gradient, determined. In this study, the mechanism of the increase of Lysozyme is discussed by using *in situ* observation.

The paramagnetic solution A mixed with $CoCl_2$ aq. of 4.5 wt.% and PEG4000 aq. of 24.0 wt.% was prepared. The solution B was Lysozyme aq. with the concentration of 80.0 g/L. Both the solution was prepared to be pH = 8.0 with TAE buffer at R. T. The 100 μL of solution A was soaked into a polystyrene made reactor with 7mmϕ, and 50 μL of solution B was stacked gently on it to form the interface. The 100 μL of liquid paraffin was stacked to prevent water from vaporizing. Then the reactor was set in a vertical magnet field up to 9.1 with the magnetic gradient. The crystal growth was observed by CCD camera from the top for 24h. The position, the size, and the growth rate of crystal were estimated.

Figure 1(a) shows a top view snapshot at around the interface between water solution and paraffin (B = 9.1 T, $B\mathrm{d}B/\mathrm{d}z$ = –587 T^2/m, t = 24 h. The size of the crystal. The crystal grew typically from 0.1 mm size at t = 3 h to 0.5 mm at t = 24 h. The crystals with few ten μL were floated up due to the reversed gravity by mean of the negative gradient magnetic field and stuck on the interface between the solvent and the paraffin as shown in Fig. 1(b). The timing of the stack was different among them. The behavior of the crystal growth was tracked for few Lysozyme crystals and the increase of the size of c-axis was plotted in Fig. 1(c). The large crystal was tend to observe if the crystal was floated up in early stage. The crystal was grown mainly on the interface just below the paraffin.

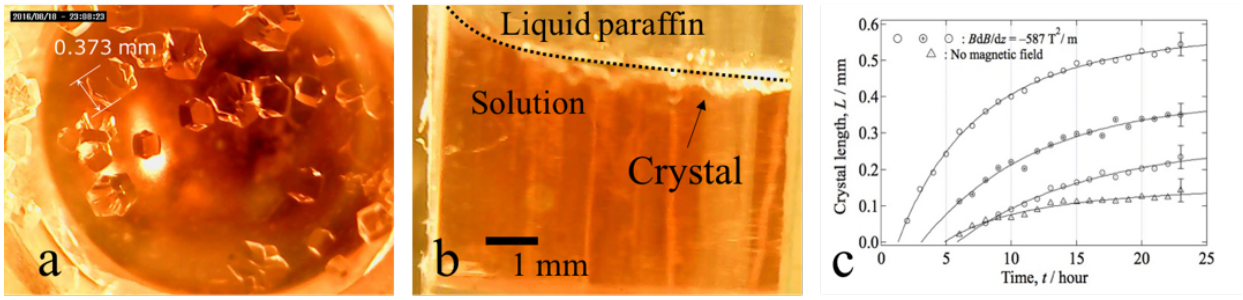

Fig. 1. (a) Top view and (b) side view of crystals on interface at t = 24 h, (c) Typical growing behavior of c-axis of crystals. All the data were observed under reversed gravity environment under vertical magnetic field with negative gradient.

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