第77回応用物理学会秋季学術講演会 講演予稿集 (2016 朱鷺メッセ (新潟県新潟市))

Crystallization of Lysozyme by LLIP Method under High Magnetic Fields Ⅱ

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The large and high quality crystal of protein is necessary to determine the function of protein by single crystal structure analysis. We have devised a crystallization technique which is used to obtain the large crystal of protein by using high magnetic field and liquid-liquid interfacial precipitation (LLIP) method. The LLIP method is one of the techniques to make a crystal. The interface between a saturated solution and a poor solvent gets supersaturated by the counter diffusion. The crystal is made in the supersaturated layer at around the 2-dimensional interface. We reported that the size and morphology of crystal were controlled by using magnetic field and LLIP; the volume of C60 rod crystal was enlarged by 100 times in reduced gravity environment with the aid of the high gradient magnetic field [1]. In our previous researches, the size of crystal of hen egg lysozyme as a typical protein was increased by LLIP under the high magnetic field [2,3]. The size effects may be caused by the specific concentration distribution due to counter diffusion and the three physical forces such as the magnetic torque, Lorentz force and Faraday force. In this study, we have investigated the mechanism of the size effect of lysozyme by *in situ* observing the growth process under the high magnetic field.

In the experiment, the mixture of poly-ethylene glycol solution and $CoCl_2$ solution was prepared as a poor solvent. Another aqueous solution of hen egg lysozyme with 80 mg/mL was also prepared. The tris-buffer was mixed both solutions to be pH = 7. The poor solvent of 100 μL was soaked into the cylindrical reactor with 7 mm diameter. The lysozyme solution of 50 μL was stacked gently on the solution to make the interface between them. Liquid paraffin of 100 μL was stacked on the lysozyme solution to prevent the evaporation. The reactor was set into the vertical bore of a superconducting magnet for 24 hours at 18.5 ℃. When the crystal grew up to the specific size, the crystal was transported and clung onto the interface between liquid paraffin and solution by Faraday force. The behavior was observed by CCD camera *in situ*.

Figure 1 shows the crystals which grew at 24 hours under the magnetic field and the drawing picture shows the process of the crystal growth. Firstly, the small crystals were obtained and clung onto the interface. Secondly, the crystals grew isotropic; the growth rate of direction of *x* axis is equal to one of *y* axis. Finally, the crystal growth of *x* direction was advanced and the crystal growth of *y* direction was stopped. The length of *x* axis of a typical crystal was 345 μm. The more details of this crystal growth will be discussed in the presentation.

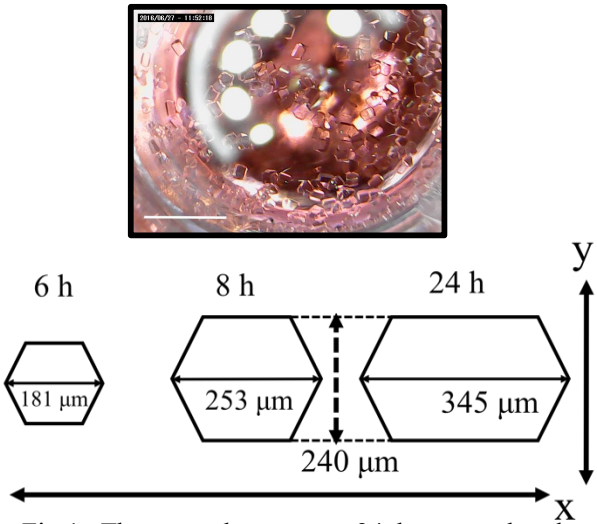

Fig.1. The crystals grew at 24 hours under the magnetic field. (white bar = 1.8 mm) The drawing picture shows the stage of the crystal growth.

Acknowledgments: This work was supported by JPSJ grant No. 16K04946.

References: [1] I. Yamamoto et al, Proc. MAP6 (2014) 26.
[2] T. Onotou, I. Yamamoto, The 62nd JSAP Spring Meeting, 11p-P1-56 (2015).
[3] T. Onotou, I. Yamamoto, Proc. MAP7 (2016).