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Impurity effect on lysozyme crystal growth under microgravity

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Microgravity is expected as an ideal condition for protein crystallization in solution, which are supported by numerous evidences such as X-ray diffraction resolutions of crystals grown in space enhanced statistically 20% comparing to terrestrial products, which was ascribed to the reduction of impurity such as dimer, foreign proteins incorporation into the crystal under microgravity condition [1]. On the other hand, it was reported that the growth rate of lysozyme crystal is equal or even larger in space environment than under terrestrial condition [2]. This result was surprising because under microgravity the buoyancy convection does not take place, which helps the mass transport of protein molecules in solution. However, why the growth of lysozyme crystal was promoted in space has been unclear. To understand the mechanism of growth promotion, step velocity of the islands on {110} face of tetragonal lysozyme crystal has been measured by the parabolic flight, which can create 20 seconds microgravity condition.

{110} faces of tetragonal lysozyme crystals, growing in 100 mg/ml lysozyme solution (Seikagaku, containing 0.5% wt. dimer) with 25 mg/ml NaCl and 50 mM sodium acetate (pH4.5), were observed in situ by a phase contrast microscopy during the parabolic flights. The temperature of growth cell was fixed during each parabolic flight. At every parabolic flights, we changed the temperature from 31 ℃ to 26 ℃ to examine the dependence on supersaturation. Correspondingly the supersaturation defined as ln(C/Ce) (C: the bulk concentration of lysozyme, Ce: solubility at a given temperature) was varied from 0.77 to 1.47. To avoid the mechanic vibration during the flight, the stage of microscopy was reinforced. Moreover the focus was adjusted by piezoelectric element under objective lens. The images of crystal surface were taken automatically every second by high resolution CCD camera and transferred to PC for further analyses. Step velocities of 2D islands on {110} faces were calculated from advancement of steps of 2D island on the lysozyme crystal surface.

The evolution of 2D islands on the lysozyme crystal surface was successfully observed during parabolic flights. In spite that microgravity condition continues for only 20 seconds, the change of step velocities before, in, and after microgravity was detected. We found that the step velocity was enhanced during microgravity in the case of ln(C/Ce) = 1.47. In addition, this enhancement lasted to the following period (supergravity condition). For cases of lower supersaturation (ln(C/Ce) = 0.77 and 1.04), there were no evidence of promotion of step velocity during microgravity. However, the step velocity seemed to be enhanced after microgravity.

These results are interpreted due to the reduction of dimer molecules, which block the movement of the growth steps [3,4], on the crystal surface. In microgravity, the concentration of dimer molecules vicinity of the crystal-solution interface decreases as crystal grows, because there is no buoyancy convection which causes efficient transport of dimer molecules from bulk solution to the interface (impurity depletion zone [5,6]). However, it was estimated that the duration of microgravity (20 seconds) was too short to cause significant depletion of dimer molecules.

Therefore, we concluded that it is difficult to explain the measured enhancement of step velocity during microgravity by the impurity depletion zone concept. Another crystal growth mechanism should exist behind the enhancement of step velocity.

References:
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