Summary report of the ISS-Kibo utilization mission, “Ice crystal growth controlled by biological macro-molecules（Ice Crystal 2）”

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Feb. 2017

The free growth of ice crystals in supercooled bulk water containing an impurity of glycoprotein, a bio-macromolecule that functions as ‘antifreeze’ in living organisms in a subzero environment [1], was observed under microgravity conditions in the Japanese Experiment Module “KIBO” of the ISS during the period from November 2013 to June 2014. The free growth experiments of H_2O ice crystals in supercooled water including an antifreeze glycoprotein (AFGP) as an impurity were conducted using a newly developed space apparatus (Ice Crystal Cell 2). The AFGP concentration in water used for these experiments was fixed at 0.07 mg/mL, and the bulk supercooling (ΔT_∞) of the water at the beginning of the crystal growth was varied between 0.1 and 0.5 K. The experiments were repeated 124 times for various supercoolings, and the growth of a polyhedral ice crystal surrounded by flat interfaces was always observed at the tip of a glass capillary inserted into the center of a growth cell filled with AFGP solution. The ice crystal growth was stably maintained in space over a period of 30 minutes (more than one hour in some cases) and observed using a Michelson interference microscope combined with a phase contrast microscope. However, an accident occurred in an operational system (Solution Crystallization Observation Facility) of ISS-KIBO shortly after the beginning of the experiments began, and the function of the phase contrast microscope was completely lost its function.　Emerging from this crisis, only the videos of the interference fringe images were acquired.

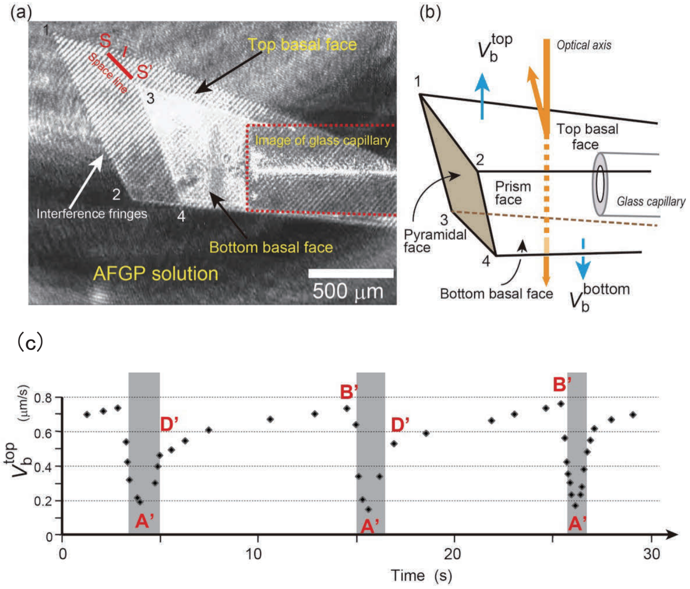

Fig. 1 (a) Interference image of ice basal face obtained at $\Delta T_\infty = 0.3\text{K}$ in ISS; (b)Three-dimensional geometry for the ice crystal; (c) Growth rates at the center of the space line as a function of growth time.

Figure 1 (a) shows a snapshot taken from a video obtained in the ISS experiments, and (b) presents the three-dimensional geometry of the ice crystal. These video images were subject to time-space plot analysis, and the growth rates of basal faces were measured as a function of growth time.　Figure 1 (c) shows the growth rates as a function of growth time. Based on these experiments, we first found the acceleration and oscillation of the normal growth rates resulting from the interfacial adsorption of these AFGP molecules, which is a newly discovered impurity effect for crystal growth.　As the convection caused by gravity may mitigate or modify this effect, secure observations of this effect were first made possible by continuous measurements of normal growth rates under long-term microgravity conditions realized only in the spacecraft.

Here we attempt to present a schematic explanation of our new findings.　First, we consider the mechanism for promoting growth of the ice basal face by AFGP molecules.　It is well known that an ice crystal growing in pure water takes on a circular disk shape surrounded by two flat basal faces and one rounded edge face [2], and that the basal faces grow by a layer-by-layer mechanism.　In contrast, the ice morphology in an AFGP solution changes to a polyhedral shape surrounded by two basal faces, six prismatic faces, and trapezoidal pyramidal faces connecting the basal and prismatic faces. AFGP molecules were thought to be adsorbed on the prismatic or pyramidal faces, but not on the basal faces, and to have the ability to smoothen the rough interfaces. These facts prompted us to speculate that the AFGP molecules are adsorbed on the edges of the growth steps on the basal face because those should be constituted by faces that include the prismatic or pyramidal faces, as illustrated in Fig. 2. As the average size of the AFGP molecules (approx. 3 nm in length) is much larger than the height of the elementary step

(0.37 nm) on the basal face, the adsorbed molecules would protrude from the growth steps and may work as sources of growth steps for the basal face. This process may thus enhance growth rates through the adsorption-promotion effect of the impurity molecules on crystal growth.

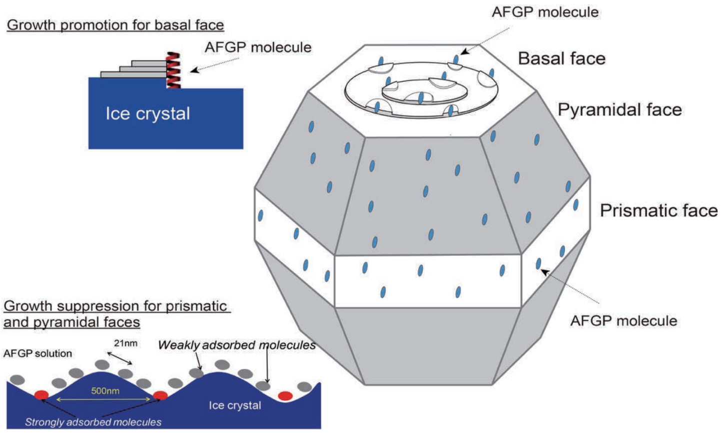

Fig. 2 Anisotropic adsorption of AFGP molecules on the basal and prism/pyramidal faces. AFGP molecules on the basal faces function as new source of growth steps.

We also consider the origin of the periodic oscillation of growth rates induced by the adsorption effect of the impurity molecules. Previously discussed models for growth oscillations have been based on strong growth depression due to the adsorption of impurity molecules at the interface, but is not applicable to the growth oscillation observed on the basal faces in our experiments. We propose a new model to explain the growth oscillation based on the adsorption-promotion effect of the impurity molecules, that is, the kinetic processes originating from the interactive variation between interfacial supercooling (i.e. the driving force) and the concentration of adsorbed impurity molecules at the interface.

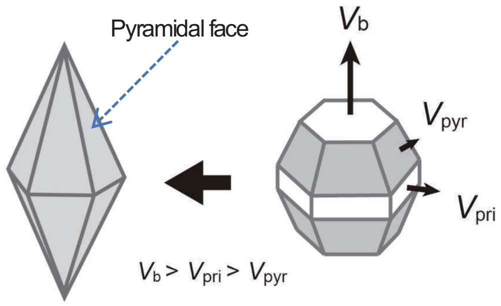

Fig. 3 Anisotropy of growth rates and crystal morphology development. Ice crystals are surrounded by only pyramidal faces having the slowest growth rates.

Finally, we note that promoting the growth of the basal face is essential to fulfilling the freezing inhibition function for living organisms under subzero conditions. Many tiny ice particles with a dodecahedral shape (several tens of micrometers in size) surrounded by only pyramidal faces (as shown in Fig. 3) are observed in the blood of fish living in a subzero environment, but do not continue to grow. The reason why the dodecahedral ice particles can stably exist in serum is explained by a key principle governing the growth of polyhedral crystals: "Flat faces with faster growth rates are truncated by the faces with slower growth rates and finally the polyhedral crystal is surrounded by only flat faces with the lowest growth rates." Consequently, the formation of dodecahedral ice particles suggests that the growth rates of the individual faces must maintain the relationship of $V_b > V_{pri} > V_{pyr}$. When all of the basal and prismatic faces on an ice crystal disappear, the ice crystals should be surrounded by only pyramidal faces having the slowest growth rates, and finally the formation of a dodecahedral ice particle is complete. This external form of an ice crystal prevents its further growth, and the water is thus kept in a supercooled state. The prevention of freezing in living organisms cannot be solely explained by the growth depression effect of AFGP molecules on ice crystals. In other words, the anisotropic functions of AFGP molecules for ice crystal growth are essential for preventing the freezing of living organisms.

Our findings will lead to a better understanding of a novel kinetic process for growth oscillation in relation to growth promotion due to the adsorption of protein molecules, and will shed light on the role of crystal growth kinetics at the onset of the mysterious antifreeze effect in living organisms, that is, how this protein may prevent fish from freezing.

References: [1] Y. Yeh and R. E. Feeney, *Chem. Rev*., 96 (1996) 601-618.; [2] C.A. Knight, et al., *Biophys. J*., 64 (1993) 252-259.; [3] S. Zepeda, et al., *Cryst. Growth Des*., 8 (2008) 3666-3672.

Main papers published in relation to this project: Oscillations and accelerations of ice crystal growth rates in microgravity in presence of antifreeze glycoprotein impurity in supercooled water, Y. Furukawa, K. Nagashima, S. Nakatsubo, I. Yoshizaki, H. Tamaru, T. Shimaoka, T. Sone, E. Yokoyama, S. Zepeda, T. Terasawa, H. Asakawa, K. Murata and G. Sazaki, *Scientific Reports*, 7:43157 (2017), doi:10.1038/srep43157.