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3. Research Fields
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4. Research Categories
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5. Research Theme
Crystal growth mechanisms associated with the macromolecules adsorbed at a growing interface and its application to growth form control

6. Investigators
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8. Summary of Research
Ice crystal growth in supercooled antifreeze protein solutions is inhibited by their adsorption at the ice/water interface, and this phenomenon is a matter of vital importance for survival of living organisms in subzero environments. Several kinds of proteins with this function are known at the present time and are mainly categorized into two types of proteins: antifreeze glycoproteins (AFGP) with sugar and antifreeze proteins (AFP) without sugar. These interactions are being studied with keen interest as the functional proteins to control ice crystal growth. While these proteins have dramatic consequence for natural biological processes and technological applications, only little is known about the dynamic mechanism of ice growth inhibition. The purpose of this research is to learn the antifreeze mechanism based on the fundamental studies of ice crystal growth.

Precise measurements of growth rates of ice crystals in AFGP solutions showed that oscillations are a fundamental feature for the crystal growth controlled by the adsorbed macro molecules such as the AFGP. Since the oscillatory phenomena may be strongly affected by the external turbulence like convection, the microgravity experiments in space will be very important to understand the mechanism of self oscillatory growth. We propose a project of a new space experiment using the ice cell specimen that has been developed by JAXA to carry out the space experiments of the morphological instability of ice crystals in pure supercooled water.

In order to observe the magnitude of the convective effects on the solution growth of ice crystals, parabolic flight microgravity experiments were conducted to precisely control the latent heat of crystallization-induced convections. When an ice crystal grows the attachment of molecules results in local heating. This produces convections within the solution, which in turn cool the surface. Under microgravity all heat-induced convection should cease, increasing the surface temperature and reducing the overall growth rates. During our experiments such results were indeed observed and quantified over a large range of temperatures.

To understand how these proteins work, we used three-dimensional confocal microscopy to gain
insight into the antifreeze interaction with the ice/solution interface. Single ice crystals are grown in solution from a capillary in the presence of antifreeze proteins labeled with fluorescein isothiocyanate (FITC). At μg/ml quantities of AFGP we could see a clear adsorption at the prismatic planes with the growth stopped while the temperature is in the hysteresis region. When we lowered the temperature below the hysteresis region growth continues while the protein is rejected from the crystal. At higher concentrations the gross morphology could vary quite dramatically, but the proteins were still rejected from the solid. Additionally, we were able to measure the adsorbed concentration of AFGPs on the stopped pyramidal planes.

In order to observe the protein distribution at the ice/water interface, one-directional ice crystal growth experiments were carried out in fluorescein isothiocyanate (FITC) labeled antifreeze protein solutions using fluorescence and phase contrast microscopies. Fluorescence images clearly show the distribution of antifreeze molecules near the growing ice/water interface. Incorporation and accumulation of AFGP molecules at the interface were clearly observed, and strongly depend on the growth rate of the ice crystal. For AFGPs, at slow growth velocities the interface is flat and the proteins accumulate at the interface and are entirely rejected from the crystal. At higher velocities the interface breaks up and forms finger structures with the proteins incorporating into the solid in veins formed between the ice fingers. From the structural correspondence between the phase contrast and fluorescence images we can deduce that no protein is trapped in the crystal matrix. In agreement with our previous experiments, this indicates a weaker adsorption than previously envisioned and contradicts previous understandings that the mechanism for antifreeze action must be a tight irreversible binding. Additionally, in this work we were able to calculate the diffusion coefficient with our technique.

A theoretical model was also developed to understand the antifreeze mechanism and self-oscillatory mechanism of ice crystals grown in antifreeze protein solutions. The fundamental feature for adsorption of these proteins is that there are two kinds of adsorption states, weak and strong adsorption. The effect of the adsorbed protein for the ice crystal growth is strongly influenced by the adsorption states. Based on this consideration, we introduce a new concept of a kinetic circle, which is the hysteresis dependence of the growth rate on the driving force.

Furthermore, we described the secondary structure of an AFGP adsorbed at the ice-solution interface, which is necessary for understanding the antifreeze mechanism of AFGPs. The attenuated total reflection (ATR)-FTIR technique described here enabled us to effectively detect the adsorbed AFGP molecules at the ice-solution interface in an AFGP/D2O solution film. During the cooling process of the solution film, the α-helical content of the AFGP increased upon freezing while no noticeable conformational changes were observed upon supercooling. Additionally, the α-helical content was maintained at a high level during the heating process from -15 to 3.8 °C under a quasi-liquid layer (QLL) on ice. The present results indicate that AFGP molecules change their conformations to α-helix under an adsorbed state at the ice-solution interface, allowing for a more effective protein/ice interaction.

Theoretical considerations for ice crystal growth in the AFGP solution were also carried out to explain the relationships between the adsorption states of antifreeze proteins and the growth kinetics.

9. Publication List


• S. Zepeda, E. Yokoyama, Y. Uda, Y. Furukawa, Direct observation of adsorption kinetics of *antifreeze glycoprotein adsorption kinetics at the ice/solution interface* (Nature pre-submission)

Patent applications

• T. Maki, S. Zepeda, Y. Furukawa, *a microscope for simultaneous phase contrast and interferometer measurements*.

10. URL
http://www.lowtem.hokudai.ac.jp/ptdice/