

## **Antifreeze protein interaction with ice/water interface**

Yoshinori Furukawa, Salvador Zepeda, and Yukihiro Uda  
Institute of Low Temperature Science, Hokkaido University,  
Sapporo, 060-0819 Japan  
*Contact e-mail: frkw@lowtem.hokudai.ac.jp*

Antifreeze proteins (AFPs) and glycoproteins (AFGPs) help fish, plants, insects, and bacteria survive sub-freezing environments. They inhibit the growth and recrystallization of ice while radically modifying the growth shapes. Our interests are in learning about the fundamental mechanism of the proteins functions by studying the ice crystal growth kinetics as well as the protein kinetics at the ice interface. We have labeled three of the fish proteins, AFPI, AFPIII, and AFGPs, with fluorescein isothiocyanate (FITC) and carried out measurements during free growth and uni-directional growth from the melt.

It is well known that these proteins function via some interface interaction with ice crystal, but the exact mechanism has eluded scientists. Surface pinning (Gibbs-Thomson) of the growing ice interface by the AF(G)Ps has been the most prominent model for the mode of action, although recently this has been challenged and modifications to the interface thermodynamics has received more attention. Aside from mutagenesis experiments directed towards examining the functional importance of specific residues, conclusions about the mechanism have been drawn from indirect studies or more precisely from studies that describe the proteins effects on the ice interface. Our aim is to address the question of the mechanism by directly studying the proteins interaction with the ice interface.

Fluorescent microscopy was used to determine interaction planes, surface concentrations as well as adsorption characteristics of AF(G)P molecules. Several key observations lead to the conclusion that the Gibbs-Thomson model does not describe antifreeze action of the AFGPs. In particular: (1) the growth forms are smooth whereas the G-T model by nature would predict a rough interface. (2) For the observed coverage, the G-T model predicts a 6.8°C freezing temperature lowering, where the largest observed is roughly 1°C. Growth resumes despite the large amounts of adsorbed proteins. (3) AFGPs do not incorporate into the ice crystal as postulated, instead they are released during re-growth.

Attenuated total reflectance (ATR)-FTIR spectroscopy was also used to determine the secondary structures of proteins vs. temperature in the liquid and solid states as well as the ice interface characteristics. The molecular dynamics simulation of ice crystal growth affected by AFP molecules provides the precise understanding the growth prohibition mechanism in molecular level. Our work helps to remove the ambiguity of the AF(G)P role in antifreeze action at the interface between ice and water.