Antifreeze proteins and antifreeze glycoproteins are a necessary tool for the survival of fish, plants, insects, and bacteria that live in sub-freezing environments. At least five distinct classes have been found that lower the freezing temperature of water, inhibit ice recrystallization, and radically modify the growth morphology of ice. Despite the wide range of compositions and structures, it has been generally understood that the AFPs function by some surface interaction. More specifically, they are thought to permanently bind to the surface and inhibit ice crystal growth by changing the surface free energy, namely the Gibbs–Thomson (GT) mechanism. Our work is aimed at directly studying the protein kinetics at the ice/solution interface by using conventional fluorescence microscopy techniques with solution crystal growth as well as one directional crystal growth schemes. We find that AFCGs, AFP, and AFGPs all adsorb at similar coverages, but the coverage predicted by the GT model is orders of magnitude larger than is observed experimentally. AFP shows a zero-negative segregation coefficient while AFCGs and AFP show no significant protein inclusions in the ice crystal matrix. Fourier transform infra-red attenuated total reflectance (FTIR-ATR) was used to determine the AFGP structure vs. temperature in the liquid and solid states as well as the ice interface characteristics. A small, but significant tightening of the flexible structure upon freezing was observed. The protein gradually approached the solution structure and this was closely correlated with the thickness of the quasi-liquid layer at the ice interface. Our work suggests that the traditional Gibbs–Thomson view does not explain the freezing suppression and the differing protein kinetics between the different classes can lead to different mechanisms. (Conflicts of interest: None declared. Source of funding: None declared.)

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11. A new look at the concentration dependence of ice-binding proteins. Yeliz Celik *, Natalya Pertaya, Junjie Liu*, Yangzhong Qin*, Di Xu*, Peter L. Davies*, Mel Braslavsky *. *Department of Physics and Astronomy, Ohio University, Athens, 45701 OH, USA; Department of Biochemistry, Queen’s University, Kingston, Ont., Canada K7L 3N6

Ice-binding proteins, IBPs, include those that have the ability to stop ice crystal growth and inhibit ice recrystallization. IBPs can be moderate or hyperactive according to their thermal hysteresis activity, which is defined as the amount of non-colligative freezing point depression. Inhibition of recrystallization and ice growth by IBPs suggest potential applications of these proteins in preventing frost damage to plants and in cryopreservation of food and organs. By using fluorescence microscopy to detect the binding planes of different IBPs to ice surfaces we have compared the interaction of hyperactive and moderately active IBPs with ice crystals. We have also developed microfluidic devices in which the solution around small ice crystals can be exchanged in a temperature-controlled environment. The experiments we performed with the novel microfluidic devices clearly showed that ice crystals were highly stabilized by bound hyperactive IBP even if the solution IBP concentration was reduced. Thus we found that thermal hysteresis is not a direct function of the concentration in the solution at the time of the measurement. Additionally, we used fluorescently tagged IBPs to demonstrate that IBPs stay on ice crystals even after the protein concentration in the solution is reduced. These results imply that protein adsorption to the ice surface is irreversible and the concentration dependence of the activity is time dependent. The techniques we have developed to investigate IBPs such as fluorescently tagged IBPs combined with microfluidics can improve the understanding of how IBPs influence ice growth. We suggest that hyperactive IBPs hold great promise for cryobiology and an understanding of their function is essential for their effective usage. (Conflicts of interest: None declared. Source of funding: The National Science Foundation (NSF) under Grant No. CHE-0848081 (co-funded by the MPS/CHE and the Molecular and Cellular Biosciences Divisions, and by the Office of International Science and Engineering and the Office of Polar Programs) and by Canadian Institutes for Health Research (CIHR) and by the Biomolecular Nanosciences and Nanoscale Technology (BNNT) at Ohio University.)


12. Recrystallization of ice crystals in sucrose solution in the presence of AFP type I. Tomoaki Sato, Kazuma Tokizawa, Takaharu Sakamoto, Kazuma Furuwata, Department of Food Science and Technology, Tokyo University of Marine Science and Technology, 4-5-7 Konan Minato, Tokyo 108-8477, Japan

Recrystallization of ice crystals is a main cause for deterioration of frozen foods during storage and distribution. Recently, many AFPs have been found in a variety of sources and have been used as additives for suppressing this recrystallization process. However, there have been few researches investigating the recrystallization behavior of ice crystals under practical storage or distribution conditions for frozen foods. In this study, the recrystallization rate of ice crystals in 25% sucrose solution, a model frozen dessert, was measured in the presence of antifreeze protein type I (AFP type 1) at −10 °C, which is relevant to practical storage or distribution temperatures of frozen desserts. The concentration of AFP type I was set to 1 µg/ml; Ice crystals during recrystallization process were observed by using a light microscope equipped with cold stage. Two microliters of sample solution enclosed between two cover slips was placed at −10 °C in a sample holder obtained from the number-averaged ice crystals, and the effect of addition of AFP type I upon the recrystallization rate was evaluated quantitatively. The recrystallization rate of the sample containing AFP type I was about 10% of that of sample without AFP. It was also smaller than that of samples containing 0.3% LBG (locust bean gum; frequently used as a stabilizer for frozen dessert to suppress recrystallization rate of ice crystals). These facts suggest 1 µg/ml AFP type I has a strong suppression effect on recrystallization of ice crystals in frozen 25% sucrose at one of practical storage or distribution conditions. (Conflicts of interest: None declared. Source of funding: The Iwatani Naoji Foundation's Research Grant.)

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Antifreeze protein (AFP) was discovered in various organisms (such as fish, insects, plants, and soil bacteria) living in cold regions. AFPs exhibits a unique ability to inhibit growth of ice crystal and causes thermal hysteresis (TH) which is a measure of strength of AFP function. It is reported that polyvinyl alcohol (PVA), which is a non-peptide compound, also exhibits a very weak TH. Here we measured THs for type III AFP from Notched-fin eelpout (Zoarces elongatus Kner) at various AFP concentrations in the presence and absence of PVA. We found that 50 mg/ml PVA increased TH to approximately 1.8 times. It was also observed that 50 mg/ml polyethylene glycol (PEG) increased TH to approximately 1.5 times. The observed THs for the mixture of AFP and PVA or PEG were higher than the sum of individual THs of them. These polymers may enhance antifreeze activity of AFP by increasing viscosity of the solution, or acting cooperatively with AFP to the ice crystal.

We further studied the structure of 0.5% agarose gel before and after freezing at −18 °C in order to estimate AFP function by using a different method from TH measurement. The gels, which contained various concentrations of AFP, PVA, and their mixtures, were frozen at −18 °C overnight and the structures were observed after thawing. We found that the gel structure was entirely collapsed after thawing with our AFP or PVA, whereas it was maintained by the addition of 0.09 mg/ml AFP or 50 mg/ml PVA. It was also found that the structure of the gel was also preserved in the presence of 0.03 mg/ml AFP and 25 mg/ml PVA. These results indicate that AFP provides a freeze-tolerance to water-containing gels and the freeze-tolerance largely correlates with AFP function. In addition, the amount of AFP which is necessary to obtain freeze-tolerance was reduced by the addition of PVA. It is assumed that freeze-tolerance is provided by the function of AFP to inhibit growth of ice crystals. Therefore enhancement of freeze-tolerance by addition of PVA suggests that AFP and PVA may cooperatively inhibit the growth of ice. (Conflicts of interest: None declared. Source of funding: None declared.)

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Cold-living bacteria possess ice nucleation proteins (INPs). INPs are considered to act as a template for heterogeneous ice nucleation and hence promote ice nucleation. INPs have attracted a great deal of attention of not only biologists but also physicists, chemists and engineers. This is because ice nucleation activities of INPs are much higher than those of well-known mineral ice-nucleators, such as silver iodide. Unfor-