

Fluorescence microscopy studies of the antifreeze proteins

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Many organisms are protected from freezing by antifreeze proteins (AFPs), which bind to ice, modify its morphology, and prevent its further growth. Since the initial discovery of AFPs in fish, they have also been found in insects, plants, bacteria and fungi. These proteins have a wide range of applications in cryomedicine, cryopreservation, and frost protection for transgenic plants and vegetables. AFPs also serve as a model for understanding biomineralization, the processes by which proteins help form bones, teeth and shells. Yet the mechanism of action of different types of antifreeze proteins is incompletely understood. Antifreeze proteins evolved independently many times with diverse structures and properties, even in closely related species. Although AFPs were discovered more than 30 years ago and have been studied extensively since then, it is not clear whether all AFPs block ice growth through a unified mechanism of action or if these diverse proteins have distinct binding properties. As measurements of the antifreeze proteins in contact with ice were elusive, this question had not been answered.

Here we are reporting on the kinetics of the interaction between AFP and ice as monitored by fluorescence microscopy for two types of AFP labeled with green fluorescent protein (GFP). This set of experiments is a new way to investigate the AFP - ice interaction. By putting a fluorescent tag on a fish AFP, we were able to directly visualize AFP binding to ice and demonstrate, by lack of recovery after photo-bleaching, that a fish AFP from ocean pout (type III) adheres irreversibly to ice surfaces. Additionally, we present the first observations of fluorescently labeled hyperactive insect antifreeze protein from spruce budworm on ice crystals. We find that differences between antifreeze protein types are manifested not only by the shape of the ice crystals but also in the way the proteins interact with the ice.

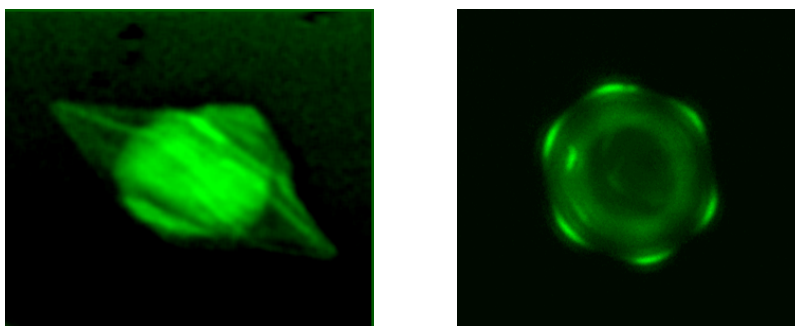


Figure 1. *Left: Confocal microscopy image of an ice crystal (20 μm) in the presence of fish antifreeze protein from ocean pout (type III) labeled with green fluorescent protein. Right: Confocal microscopy image of the ice crystals (15 μm) in the presence of the insect spruce budworm AFP labeled with green fluorescent protein.*

For example, ice in a solution of GFP-labeled AFP type III at low temperature grows as fine needles that become covered with protein, resulting in a bright core. These crystals retain fluorescent AFPs within the original core, and newly formed bipyramidal surfaces that emerge in moderate supercooling become coated with additional AFPs. In contrast, GFP-labeled spruce budworm AFP does not form a bright core but accumulates on the corners of a hexagonal ice crystal.

These results, which show directly the position of AFP on ice, pave the way for a better understanding of AFP activity. The system of AFPs and ice can be used as a model platform to understand bio-mineralization processes and thus is important for future nanotechnology applications. Because unrelated AFPs with diverse structures and properties have evolved independently, we speculate that if there are life forms that have evolved in the presence of ice on planets other than Earth, it is possible that they have evolved antifreeze molecules and thus antifreeze activity might be one indicator for the presence of life.

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