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Crystallization of ice in AFP-III solutions

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The purpose of our research is to explore how type III AFP modifies the morphology and growth kinetics of ice crystals, and to reveal the action mechanisms of AFP-III.
In a glass capillary seed crystals of ice of hexagonal modification were formed in supercooled water with AFP-III concentrations of 0-0.8 mg/ml. Measurements of growth rates were performed by Mach-Zehnder interferometry at supercoolings from 0 to 1.0 K. We have found that AFP-III exhibits an extremely high activity. Even at a concentration of 0.001 mg/ml it changes the morphology of ice crystals, and prismatic faces appear in the a-axis directions instead of circular disk-like shapes typical for growth in pure water. Our measurements of the growth rates have shown that AFP-III molecules can act both as an inhibitor for the prismatic faces and as a promoter for the basal faces of ice crystals, and cause the formation of macrosteps on the basal faces. The value of growth thermal hysteresis rises non-linear with the increase in AFP-III concentration. The influence of AFP-III on the crystallization of ice was explained by adsorption of the protein molecules on the crystal surface. Retardation of ice growth in the presence of AFP-III occurs due to blocking of the surface by adsorbed protein molecules. From thermodynamics, it can be expected that an ice surface covered by the protein molecules will be able to grow when the inter-molecule distance exceeds the critical value predicted by the Gibbs-Thomson law. Direct observations by confocal fluorescent microscopy of the protein molecules labeled with a fluorescent isocyanate allowed to estimate the average distance between AFP-III adsorbed on the prismatic surface of ice crystals. The theoretical values of the freezing point depression in the presence of AFP-III corresponded with the experimental data when we assumed that AFP-III decreased the surface free energy of the faces of the ice crystal.
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Significance statement

Antifreeze proteins (AFPs) exhibit a strong ability to control the nucleation and growth of ice. The inhibition effect of AFPs on the recrystallization and growth of ice is very common in nature, and it helps living organisms (sea fish, plants, insects) to survive in a subfreezing environment.

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