Enhancing the Activity of Hyperactive Antifreeze Proteins with Additives

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The unique ability of antifreeze proteins (AFPs) to inhibit ice growth and recrystallization makes them excellent potential cryo-protectants in cryopreservation of food, cells and organs, cryosurgery, food engineering, and agriculture. AFPs can be classified as hyperactive (hypAFPs) or moderate AFPs according to their thermal hysteresis (TH) activity, which is defined as the difference between melting temperature and non-equilibrium freezing temperature. While moderate AFPs are already used in the food industry, to the best of our knowledge, the hypAFPs have not been utilized for applications outside of basic science research. One of the main reasons is that the production of hypAFPs is prohibitively expensive and currently inefficient compared to moderate AFPs and other cryo-protectants. In order to facilitate the transition of hypAFPs from the lab to industry, we intend to study the efficient production of hypAFPs, their use in cryopreservation methods, and the enhancement of their activities. It has been suggested that, in nature, AFPs work in synergy with other factors in the hemolymph of the organisms, and it was found that some materials enhance their activity. Here we discuss our recent results of combinations of AFPs with other substances to achieve higher TH levels. Using a nanoliter osmometer, we examined the influence of materials such as disaccharides and synthetic polymers on the TH of hypAFPs. Disaccharides lower the melting temperature as expected from the increased osmolarity, but they reduce the non-equilibrium freezing temperature even more. We found that a solution containing few µM of hypAFP, *Tenebrio molitor* AFP (*Tm*AFP), gave up to three-fold higher TH activity when combined with 1 M sucrose or trehalose. We also found that polyvinyl alcohol (PVA) at a concentration of few mM influence the TH of *Tm*AFP, but much more significantly was the combination of PVA and disaccharides that gave high TH, up to seven times the TH of *Tm*AFP by itself. The use of additives to enhance the activity of hypAFPs shows great promise and may lead to the development of improved cryoprotectants.

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