How hyperactive antifreeze proteins relate to 'regular' ice-binding proteins

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Characterization of insect antifreeze proteins (AFPs) showed that they were an order of magnitude more active than the typical fish AFP. This led to them being called "hyperactive" and to their discovery in other biological kingdoms. A consistent property of hyperactive AFPs is that they prevent ice crystal growth along the *c*-axis. Thus, when the freezing hysteresis is exceeded and an ice crystal grows rapidly, it does so along the *a*-axes when in the presence of hyperactive AFPs, and along the *c*-axis when the crystal is bounded by "regular" AFPs. This diagnostic difference correlates with the ability of an AFP to bind to, and block growth from, the basal plane of ice. Using traditional ice-etching analysis (and/or a modification thereof where the AFPs are fluorescently labeled and visualized without sublimation) we have repeatedly shown that all hyperactive AFPs bind to the basal plane of ice, and that regular AFPs do not bind this ice plane.

To date, the only hyperactive AFP found in fishes is the hyperactive type I AFP (type Ih) from flounders. The protein is a 33-kDa dimer of two very long, 195-residue alpha-helices. Each helix has the same alanine richness and similar 11-residue repeats as the small type I AFPs from skin and plasma. Type Ih is present at low levels in flounder plasma. The protein is very thermolabile and irreversibly denatures above 18 ºC. We have developed a method to purify the protein in amounts needed for X-ray crystallography. Structural determination of this protein will elucidate the unusual way in which these helices dimerize. It will also potentially explain how the larger protein binds to many ice planes, including the basal plane, and has thereby achieved hyperactivity, when it is clearly homologous to smaller proteins that only bind to a single plane of ice.

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