Isolation and Ice binding properties of antifreeze proteins from polar regions.

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Ice-binding proteins (IBPs) inhibit ice growth through direct interaction with ice crystals that permit the survival of polar organisms in extremely cold environments. FfIBP is an ice-binding protein encoded by the Antarctic bacterium *Flavobacteriumfrigoris*PS1. The refined structure of FfIBP shows an intramolecular disulfide bond, and analytical ultra-centrifugation and analytical size exclusion chromatography show that it behaves as a monomer in solution. Sequence alignments and structural comparisons of IBPs allowed us to define two groups within IBPs, depending on sequence differences between the α2 and α4 loop regions and the presence of the disulfide bond. Although FfIBP closely resembles *Leucosporidium* IBP (LeIBP) in its amino acid sequence, the thermal hysteresis (TH) activity of FfIBP appears to be 10-fold higher than that of LeIBP. A comparison of the FfIBP and LeIBP structures reveals that FfIBP has different ice-binding residues as well as greater surface area in the ice-binding site. Notably, the ice-binding site of FfIBP is composed of the T-X-A/G-T/N motif, which is similar to the ice-binding residues of hyperactive antifreeze proteins. Thus, we propose that the TH activity difference between FfIBP and LeIBP may come from the amino acid composition of the ice-binding site, which correlates with differences in affinity and surface complementarity with the ice crystal.

Keywords: antifreeze protein, ice-binding protein, FfIBP, *Flavobacteriumfrigoris*PS1, X-ray crystallography