The role of dynamics in the structure and function of antifreeze proteins

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The type I antifreeze protein, HPLC6 isoform, is 37 residues long and consists of a single α-helix. The simplicity of its sequence and structure has led to this protein being a model for all antifreeze proteins (AFPs). The C-terminus of the protein is capped with an amide group; its absence has been shown to result in a 30% loss of thermal hysteretic activity. Starting with an Ala37 mutant and a SUMO N-terminal tag, we use SUMO protease and carboxypeptidase-Y with arginamide to create a bacterial recombinant type I AFP that is completely indistinguishable from type I AFP from winter flounder in terms of its molecular weight, structure and activity. Using NMR relaxation experiments and circular dichroism, we show that the C-terminal amide is important for maintaining a rigid C-terminal structure and full activity, but its absence does not decrease the amount of α-helicity. We also show that the C-terminal arginine side chain is not important for maintaining rigidity, but may instead play a role in keeping the protein highly soluble. A potentially extreme example of dynamics in antifreeze protein activity is that of plant dehydrins, a family of intrinsically disordered proteins. Dehydrin PCA60 extracted from peach bark was previously shown to have weak thermal hysteresis and the ability to shape ice crystals. Our measurements with recombinant PCA60, the dehydrin Dhn5 from winter rye, and synthetic large dehydrins from wild grape failed to show any TH activity or ice-recrystallization activity. This suggests that the recombinant form may lack a post-translational modification, or that a co-purified protein may be responsible for the observed antifreeze protein activity.

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