Exploring the activity-size relationship in IBPs through protein engineering

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Abstract

Antifreeze proteins (AFPs) bind ice to prevent its growth. The binding mechanism is not established, but one hypothesis proposes that AFPs arrange ice-like waters on their ice-binding site (IBS) that merge with, and freeze to, the ice lattice. The activity of AFPs is measured by the difference between the slightly elevated melting and depressed freezing points and is termed thermal hysteresis (TH). In natural AFP isoforms, there is an exponential positive relationship between the surface area of the IBS and its TH activity. Here, we explored this relationship by engineering the structure of an AFP from the snow flea, *Granisotoma rainieri* (*Gr*AFP). *Gr*AFP is a nine polyproline type II helical bundle protein where the helices are arranged into two layers. One layer has a relatively hydrophobic, flat face, which is thought to be the IBS, while the other is irregular and charged. Mutations were made on both sides of the AFP to identify the IBS. A single Lys substitution on the hydrophobic face reduced TH activity to 10%, while the double mutant had no TH activity. Engineered constructs with one fewer and one more helix on the IBS (25% surface area change) had a 10-fold decrease and 2-fold increase, respectively. Current engineering experiments are attempting to also increase the length of the IBS to broaden the understanding of structure-function relationships in AFPs and help explore the upper limits of antifreeze activity.

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