Engineering a naturally inactive SP-like isoform of type III AFP into a fully-active QAE-like isoform

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Type III antifreeze protein (AFP) is produced by several species of marine fishes that inhabit ice-laden waters. This moderately-active, 7-kDa, globular AFP is produced *in vivo* as a mixture of QAE-Sephadex-binding and SP-Sephadex-binding isoforms. QAE isoforms can be further divided into QAE1 and QAE2 groups based upon their amino acid conservation. Members of the QAE1 family are fully active and capable of binding two or more planes of ice, while QAE2 and SP isoforms can not prevent ice growth and adsorb only to a pyramidal plane of ice. Here we describe the conversion of an 'inactive' QAE2 isoform (nfeAFP11) from the Japanese Notched-fin Eelpout (*Zoarces elongatus*) into a fully-active QAE1 isoform. Four mutations total (V9Q, V19L, G20V, I41V) were required to endow nfeAFP11 with thermal hysteresis activity equivalent to that of nfeAFP8, a fully-active QAE1 isoform from the same fish. Ice crystal morphology and target ice plane specificity, as monitored via fluorescence-based ice plane affinity (FIPA) analysis, reflected the gain in activity with each successive mutation that formed the prism-binding site. Wild-type nfeAFP11 produced hexagonal trapezohedrons and adsorbed solely to a pyramidal plane of ice, while the fully-active nfeAFP11 tetra-mutant produced hexagonal bipyramids and bound to both the primary prism and a pyramidal plane of ice. Differences in ice-plane specificity between the various mutants of nfeAFP11 tested in this study correlate with differences in the location of anchored clathrate waters on their IBSs as shown by molecular dynamics simulations.

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