NMR Studies of the Snow Flea Antifreeze Protein as a Model Polyproline II Helical Bundle to Advance Protein Design

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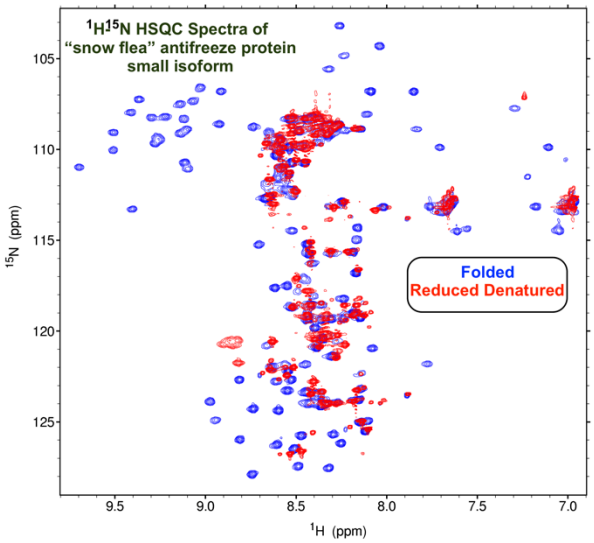
The *Hypogastrura harveyi* antifreeze protein was discovered by Graham and Davies in Canadian "snow fleas" in 2005 (1). Despite a remarkably high content of glycine residues, the small isoform of this protein adopts a stable fold consisting of six polyproline II (PPII) helices (2). Recently, several additional proteins or domains formed by a bundle of interlinked glycine-rich PPII helices has emerged (3). These proteins adopt remarkably flat brick-like or honeycomb forms with unique structural properties, but the basis of their conformational stability is unclear. Protein Design has made enormous strides in the last decade (4). Based chiefly on α-helices and β-sheets, this field mostly ignores PPII helices, the third class of protein secondary structure. To help address this shortcoming, we have studied the conformational, stability and dynamics of folded *H. harveyi* "snow flea" antifreeze protein (sfAFP) using NMR spectroscopy and computational tools (5). Twenty-eight glycine residues show remarkably different ^{1}Hα2 versus ^{1}Hα3 chemical shift values which arise from weak Cα-H···O=C hydrogen bonds. Two disulfide bonds, an extensive network of interhelical N-H···O=C hydrogen bonds plus hydrophobic surface burial upon dimerization afford additional stability. Using an unconventional assignment approach based on sequential ^{13}C', ^{15}N and ^{1}HN connectivities (6), we recently assigned the reduced, denatured state of sfAFP and performed ^{15}N relaxation analyses, namely {^{1}H}-^{15}N NOE, R_1 and $R_{1\rho}$, under three different conditions (7). Despite its high percentage of intrinsically flexible glycine residues, sfAFP's folded and denatured states have similar mobilities as those of typical globular proteins. This implies that the free energy cost of losing conformational entropy upon folding will also be similar. This knowledge should facilitate using PPII helices as a building block for Protein Design.



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