Fungal Antifreeze Protein Consists of a Unique β-Solenoid Structure

We solved the first crystal structure of a 223-residue monomeric antifreeze protein (AFP) from snow mold fungus, *Typhura ishikariensis* (*Tis*AFP6). It forms a semipear-shaped globular structure, whose principal constituent is a 6-loop right-handed β-solenoid with a triangular cross-section, in which the N- and C-terminal loops are uniquely adjacent to each other within the solenoid. The sequence of the loops exhibited no repetitive property, whose lengths are ranging from 18 to 27 residues. This β-solenoid locates a putative ice-binding site in one of the three faces of *Tis*AFP6, whose irregularity is remarkable compared with that identified in the known AFPs.

AFP is a macromolecule that specifically binds to an ice crystal to inhibit its growth, facilitating the freeze tolerance of AFP-containing organisms such as fishes, insects, bacteria, and fungi living in cold environments. We have examined the biochemical property and the ice-binding activity of two isoforms of 223-residue fungal AFP from *Typhura ishikariensis* (*Tis*AFP6 and 8) [1]. The primary sequences of the two *Tis*AFP isoforms exhibited no similarity to that of "hyperactive AFPs" from insects and bacteria consisting of tandem repeat sequences. Nevertheless, the two fungal AFP isoforms were capable of binding to both prism and basal planes of an ice crystal similarly to the hyperactive AFPs. Here we determined the 0.95-Å high-resolution crystal structure of *Tis*AFP6, and characterized its ice-binding site (IBS) [2]. The *Tis*AFP6 isoform constructs a 52-Å-long semipear-shaped structure, whose principal constituent is a six-loop right-handed β-helix (β-solenoid) having a triangular cross-section as well as a 20-residue α-helix that lies alongside and parallel to the β-solenoid. A prominent feature of this β-solenoid is that the β1 loop or coil originating from near the N-terminus lies adjacent to the β6 loop from the C-terminus. In this way, the terminal loops are side by side within the parallel β-helix as illustrated by the spectrum colors showing blue next to red (Fig. 1). The six loops consist of no repetitive sequence, and their lengths range from 18 to 27 residues. The three 18-residue loops (β1, β6, and β5) are consecutively arrayed at the top of the molecule, while the bottom loops (β4, β3, and β2) have 3- to 9-residue insertions that extend outward on the same side to form the pear-like shape.

The β-helical fold of *Tis*AFP6 is stabilized by the following elements: (i) The hydrogen-bonding network between peptide CO and NH groups parallel to the β-helical axis. (ii) The cap structures stabilizing the edges of the β-strands in the terminal loops β1 and β2 to cover a hydrophobic core constructed in the β-solenoid. Note that the capping structures normally reside at the N- and C termini of a β-solenoid protein, but in *Tis*AFP6 they are formed internally between β1 and the α-helix and between the α-helix and β2. (iii) Approximately 48 aliphatic and aromatic residues construct the hydrophobic core without the support of disulfide bonds. (iv) The long α-helix that runs the length of the β-solenoid. There are 12 hydrophobic residues at the interface between the α-helix and β-solenoid.

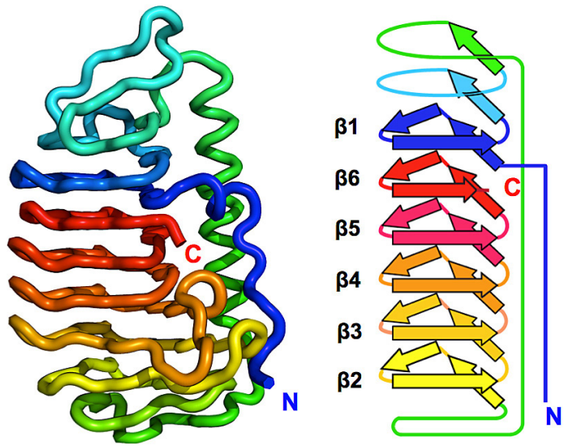

Figure 1: Backbone structure and its schematic diagram of *Tis*AFP6 to represent the construction of an irregularly ordered β-solenoid, in which the N- and C-terminal loops are side by side.

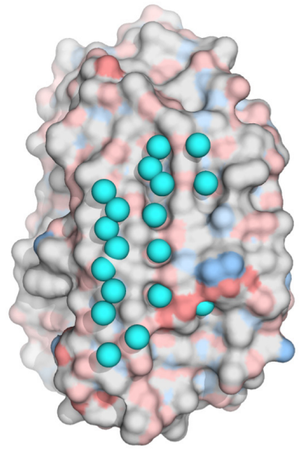

Figure 2: A selected surface of *Tis*AFP6 that locates an ice-binding site (IBS), in which the water molecules are immobilized in a few grooves. They are thought to anchor the AFP-ice interaction.

The IBS of *Tis*AFP6 was examined by steric mutations in which a tyrosyl residue replaced a short-chain amino acid. The antifreeze activity of mutants T82Y, N178Y, and V221Y, where the substitutions appeared in the middle of the three faces of the triangle, was compared with that of wild-type *Tis*AFP6. The data clearly showed that the N178Y mutation caused a significant (60%) loss in the activity. In contrast, T82Y and V221Y lost only 15% of the wild-type activity. With the help of fluorescence-based ice plane affinity (so-called FIPA) analysis of these mutants, we determined that one of the molecular surfaces including N178 locates a relatively flat IBS. Interestingly, this IBS had irregularly arrayed surface-bound water molecules (Fig. 2), which is probably due to the irregularly-ordered β-solenoid of *Tis*AFP6, which is completely distinct from the known hyperactive AFPs that locate regularly arrayed water molecules on their tandem repeat sequence. The water molecules were thought to share the positions of the ice lattice at the moment AFP attaches onto ice. A comparison of the primary sequence between the AFPs reported for various microorganisms suggests that the above-described structural features might be adopted in many other fungal AFPs.

REFERENCES

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BEAMLINES

BL-5A, BL-17A and AR-NW12A

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