Structure-based characterization and antifreeze properties of a hyperactive ice-binding protein (FfIBP) from the Antarctic bacterium *Flavobacterium frigoris* PS1

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Abstract

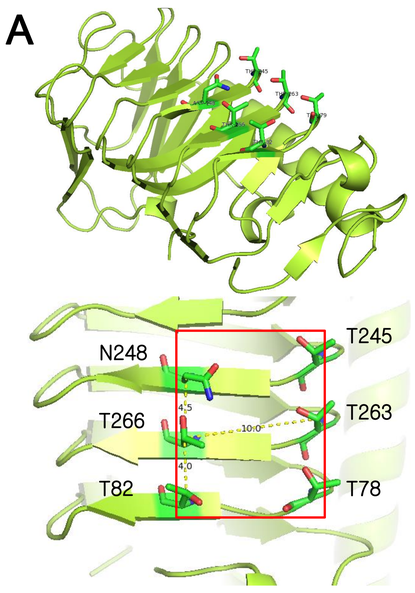
Ice-binding proteins (IBPs) inhibit ice growth through direct interaction with ice crystals to permit the survival of polar organisms in extremely cold environments. FfIBP is an ice- binding protein encoded by the Antarctic bacterium *Flavobacterium frigoris* PS1. The X-ray crystal structure of FfIBP was determined to 2.1 Å resolution to gain insight into its ice- binding mechanism. The refined structure of FfIBP shows an intramolecular disulfide bond, and analytical ultracentrifugation and analytical size-exclusion chromatography show that it behaves as a monomer in solution. Sequence alignments and structural comparisons of IBPs allowed two groups of IBPs to be defined, depending on sequence differences between the β2 and β4 loop regions and the presence of the disulfide bond. Although FfIBP closely resembles *Leucosporidium* (recently re-classified as *Glaciozyma*) IBP (LeIBP) in its amino-acid sequence, the thermal hysteresis (TH) activity of FfIBP appears to be ten-fold higher than that of LeIBP. A comparison of the FfIBP and LeIBP structures reveals that FfIBP has different ice-binding residues as well as a greater surface area in the ice-binding site. Notably, the ice-binding site of FfIBP is composed of a T-A/G-X-T/N motif, which is similar to the ice-binding residues of hyperactive antifreeze proteins. Thus, it is proposed that the difference in TH activity between FfIBP and LeIBP may arise from the amino-acid composition of the ice-binding site, which correlates with differences in affinity and surface complementarity to the ice crystal.

Introduction

Many plants, insects, animals and other organisms have evolved unique adaptive mechanisms that allow them to survive in harsh environments with extreme temperatures (Rothschild & Mancinelli, 2001). To survive in extremely cold environments, many organisms produce ice-binding proteins (IBPs). These proteins can lower the freezing point of a solution non-colligatively without affecting the melting point of the solution (thermal hysteresis); thus, the freezing of body fluids can be prevented. In addition, these proteins can inhibit ice re-crystallization, where large ice crystals grow at the expense of smaller ones, thus preventing cell damage during freeze–thaw cycles (Knight, DeVries, et al., 1984, Knight et al., 1991). It is generally accepted that these proteins function through adsorption of their flat ice-binding surfaces onto particular planes of ice crystals, thus preventing or inhibiting further ice growth (Yeh & Feeney, 1996). Notably, the antifreeze activities of antifreeze proteins (AFPs) and IBPs vary and depend on the protein structure. Therefore, structural studies are essential to understand the antifreeze and ice-binding mechanisms of these proteins.

New ice-binding proteins (IBPs) have been identified in Antarctic sea-ice algae (Raymond et al., 2009), fungi (Hoshino et al., 2003, Xiao et al., 2010, Kondo et al., 2012), mushrooms (Raymond & Janech, 2009), bacteria (Raymond et al., 2007, Do et al., 2012) and the Arctic yeast *Leucosporidium* sp. AY30 (Lee et al., 2010, Park et al., 2012, Park et al., 2011). Recently, the crystal structure of IBP from *Leucosporidium* sp. AY30 (LeIBP) was determined in our laboratory (Lee et al., 2012). Structural and functional studies of LeIBP have shown that it has a β-helical fold similar to those of insect and bacterial AFPs (Lee et al., 2012). Another IBP (FfIBP) from the Antarctic bacterium *Flavobacterium frigoris* PS1 has recently been identified from sea ice on the shore of McMurdo Sound (GenBank accession No. AHKF00000000.1; (Raymond & Kim, 2012) and characterized (Do et al., 2012). FfIBP shows 56% sequence similarity to LeIBP, but it has an up to ten-fold higher antifreeze activity than LeIBP. In this study, we compared this structure with other known structures in order to describe their hyper activity.

Results



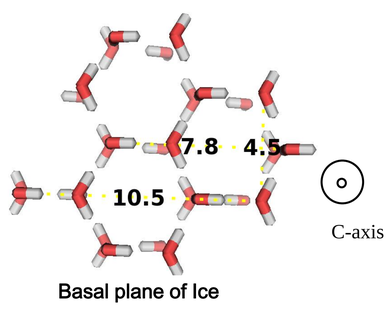


Fig 1. Ice binding site (IBS) of FfIBP. FfIBP has T-A(G)-x-T/N on IBS. x indicates hydrophobic residue pointing at inside of molecule. Distances between O atoms are indicated in angstroms. The distance between T and T or N is around 10Å, which is similar to the distribution of oxygen atoms of basal plane ice.

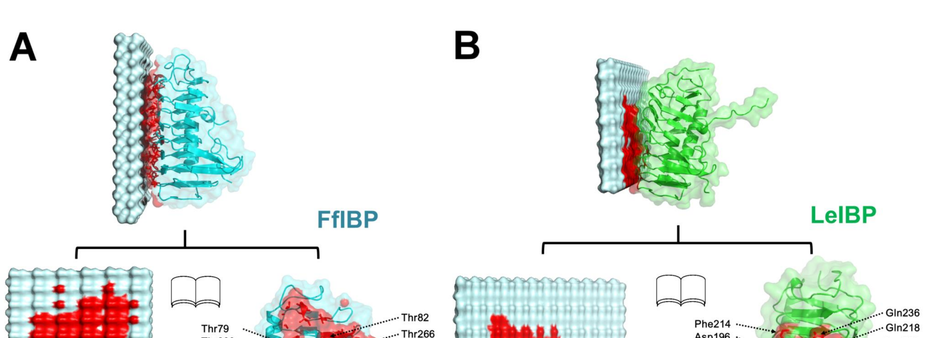


Fig 2. Comparison of the ice-binding interface area of FfIBP and LeIBP (A) Open-book view of the ice-binding interface of FfIBP. The solvent-accessible molecular surface of FfIBP is shown semi-transparently over a representation of their secondary structure to illustrate the complementarity of the binding surfaces. The ice-binding surface of FfIBP is colored red. (B) Open-book view of the ice-binding interface of LeIBP. The ice-binding interface of LeIBP is shown in pink. The ice-binding interface of FfIBP has a larger surface area (~620 Å^2) with good shape and complementarities than that (~410 Å^2) of LeIBP.

Results

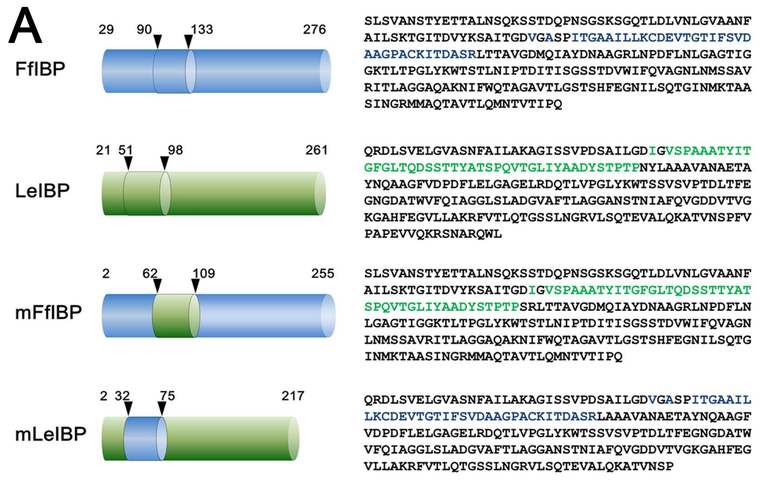
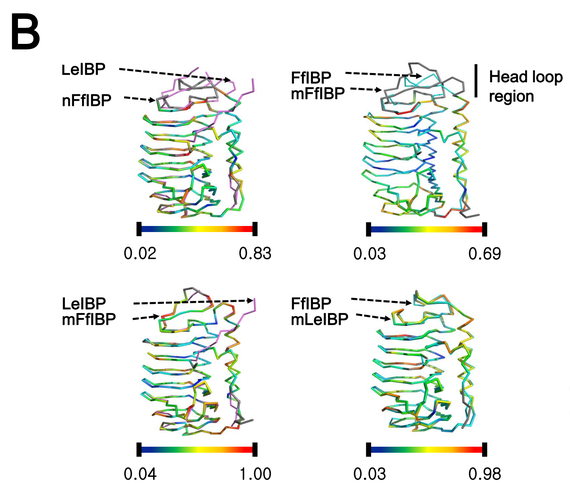


Fig 3. Capping loop structure of mFfIBP and mLeIBP. (a) Schematic diagram and amino-acid sequences of FfIBP, LeIBP, mFfIBP and mLeIBP. Two chimeric proteins (mFfIBP and mLeIBP) were constructed in which the α4 - α5 connecting loop sequences (blue or green) were swapped. (b) Structural comparison between LeIBP, mLeIBP , mFfIBP and FfIBP.



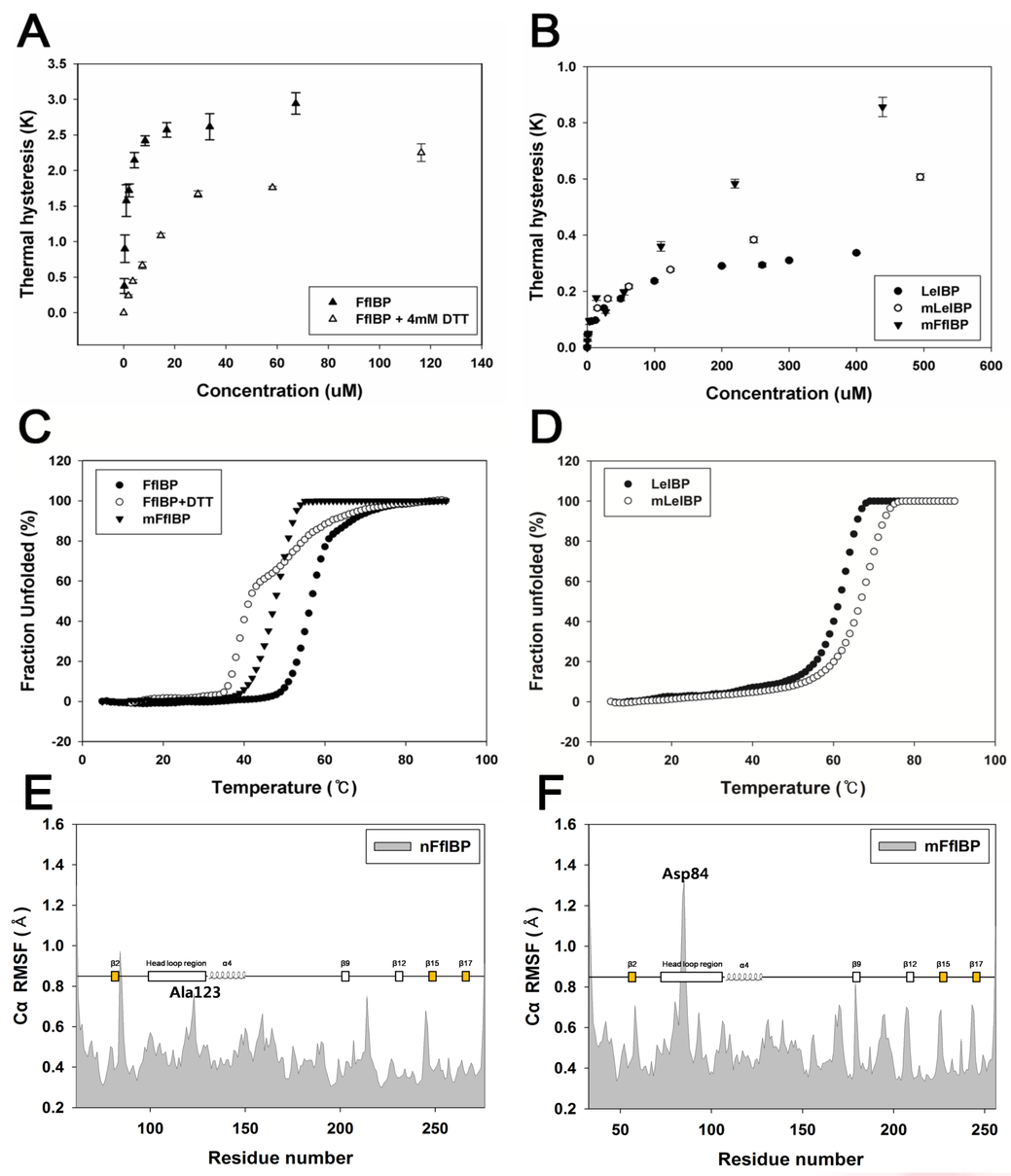


Fig 4. TH activity and thermal stability of IBPs. (a) TH activity curve of FfIBP in the absence and presence of 4 mM DTT. (b) TH activity of LeIBP, mLeIBP and mFfIBP. Each point was measured three times and averaged. (c) CD spectra demonstrating the thermal denaturation of FfIBP (filled circles), FfIBP with 4 mM DTT (open circle) and mFfIBP (filled triangle). (d) CD spectra demonstrating the thermal denaturation of LeIBP (filled circles) and mLeIBP (open circles). (e and f) Residual Ca RMSF of FfIBP and mFfIBP during the simulation. The backbone fluctuation increases in case of mFfIBP compare to the wild-type FfIBP at head loop region as well as β-strands. The long rectangular box indicate the head loop region. The β-strands at ice binding surface are shown in yellow box.

Results & Conclusion

I. FfIBP at least binds to the basal plane of ice.
II. The T-A(G)-x-T/N motif of FfIBP is well matched with the geometric lattice of water molecules of ice
III. The broad range adhesion of FfIBP to ice could be a cause of the increase in activity.
IV. FfIBP is stabilized by the different folding and disulfide bond on the head loop region.
V. Molecular dynamics revealed that the head loop region is also important for the maintenance of hyperactivity in FfIBP

Reference

Do, H., Kim, S-J., Kim, H. & Lee, J. (2014). Structure-based characterization and antifreeze properties of an hyper active ice-binding protein from Antarctic bacteria, Flavobacterium frigoris PS1. *Acta Crystallographica Section D: Biological Crystallography*.