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General Session

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Isolation and characterization of cysteine-rich ice-binding protein secreted from Antarctic microalga, *Chloromonas* sp.

Woongsic Jung[1,2], Youn-Ho Gwak[1] and EonSeon Jin[1]

[1]*Department of Life Science, College of Natural Sciences, Hanyang University, Seoul, Korea*

[2]*Division of Polar Life Sciences, Korea Polar Research Institute, KIOST, Incheon, Korea*

The freeze-tolerant microorganisms in Antarctic regions have produced ice-binding proteins (IBPs) to prevent freezing status and to survive from cold environment. The IBP from Antarctic freshwater microalga, *Chloromonas* sp., was identified and characterized. The length of the gene of *Chloromonas* sp. IBP (ChloroIBP) was analyzed to be 993 bps and the molecular weights deduced from cDNA of ChloroIBP were estimated to be 34.0 kDa. The expression of ChloroIBP gene was analyzed to be up-regulated and down-regulated on the thermal and freezing conditions, respectively. Western blot analysis demonstrated that native ChloroIBP was completely secreted into the culture medium. The cross-linking among fifteen Cys residues of ChloroIBP was revealed to influence the in-gel mobility according to the redox condition. The open-reading frame (ORF) of ChloroIBP was cloned and over-expressed in *Escherichia coli* to characterize the ice-binding activity and to measure the depression of the freezing points below the melting points (thermal hysteresis, TH). Recombinant ChloroIBP (Trx-ChloroIBP) purified by affinity chromatography was investigated to show a dendritic shape of single ice crystals with a TH activity of 0.4±0.02 °Cattheconcentrationof5mg/ml.Fromthe homology modeling, predicted three-dimensional protein structure of ChloroIBP was analyzed to be a right-handed β-helix consisted of three distinct β-sheet faces. A conserved region of six T-X-T motifs predicted as ice-binding moieties was located on the b-2 face and was proven to be a region bound to ice surface by PCR-based site-directed mutagenesis. In addition, it was proposed that hydrophobic interactions between hydrophobic residues on b-1 and b-2 face, the region of ice-binding motifs, were critical to maintain the structural topology of ice-binding motifs and ice-binding activity of ChloroIBP.