

Interactions of Type I AFP Side Chains with Water and the Involved Molecular Dynamics at the AFP-Ice Interface

Yong Ba and Yougang Mao

Department of Chemistry and Biochemistry, California State University Los Angeles, Los Angeles, California 90032. E-mail: yba@calstatela.edu.

Antifreeze proteins are able to inhibit the growth of seed ice crystals through binding to specific ice surfaces. To understand the binding mechanism at molecular level, we have carried out ^{13}C relaxation and ^{13}C - ^{15}N dynamic REDOR (Rotational Echo DOuble Resonance) solid state NMR at low temperatures to study the molecular interactions of selected side chains in the HPLC6 isoform of type I AFP with water and the involved molecular dynamics at the AFP-ice interface. The primary sequence of the type I AFP consists of three 11-residue repeat units commencing with the threonine residues and its secondary structure is of α -helix. Antifreeze activity studies through site-directed mutageneses by Davies group suggest that the ice-binding surface of type I AFP comprises the Thr side chains and the conserved i+4 and i+8 Ala side chains, where i denotes the positions of the Thrs along the primary sequence. To find direct structural evidence for the AFP's ice-binding surface, we have carried out the solid state NMR experiments on three site-specific ^{13}C and ^{15}N isotopically labeled type I AFPs including: (1) ^{13}C labeled at the periodically equivalent 17th and 21st Ala side chains (i+4, 8); (2) ^{13}C labeled at the periodically equivalent 8th, 19th and 30th Ala side chains (i+6); and (3) $^{13}\text{C}_\gamma$ and ^{15}N doubly labeled type I AFP at the periodically equivalent i=13 and 24 Thr residues. Model of Ala methyl group C-C chemical bond rotation/three-site rotational jump together with that of water molecular reorientation was proposed to calculate the interactions and dynamics. Analyses of the T_1 data show that water molecules in the ice surface closely capped the i+4 and i+8 methyl groups within van der Waals interaction range while the surrounding water molecules to the i+6 methyl groups could be looser or consisted of translational motions. Similar interaction as in the (i+4, 8) AFP was also found for the Thr methyl groups but with longer average van der Waals interaction range. Correlation times of the rotational/reorientational motions of the involved methyl groups and water and the corresponding activation energies were also obtained from the theoretical analyses. Analysis of the dynamic REDOR data of the Thr $^{13}\text{C}_\gamma$ and ^{15}N doubly labeled type I AFP shows that when the AFP interacted with the ice surface in the frozen AFP solution, the conformations of the Thr side chains changed from all anti conformations as in the AFP crystal structure to partial population in the anti conformation and partial populations in the two gauche conformations. This change together with the structural analysis indicates that simultaneous interactions of the methyl groups and the hydroxyl groups of the Thr side chains with the ice surface could be the underlying reason.

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