A hyperactive, heat-stable antifreeze protein from an Antarctic sea ice bacterium

Yuichi Hanada[1), 2)], Hidemasa Kondo[1), 2)], Christopher P. Garnham[3)], Seiya Togashi [4), 2)], Yoshiyuki Nishimiya[2)], Tamotsu Hoshino[1), 2)], Ai Miura[2)], Peter L. Davies[3)], Sakae Tsuda[1), 2)]

[1)] Graduate School of Lifescience, Hokkaido University, Sapporo, Japan

[2)] Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Sapporo, Japan

[3)] Department of Biochemistry, Queen's University, Kingston, Ontario, Canada K7L 3N6

[4)] School of Science, Hokkaido University, Sapporo, Japan

Antifreeze proteins (AFPs) are characterized by their unique ability to prevent ice crystals from growing. Recently, AFPs sharing more than 40% sequence identity at the amino acid level were found in very divergent taxa, including fungi, bacteria, diatoms and copepods. These related AFPs are thought to play a crucial role in the adaptation to cold environments, and we are therefore interested in studying their structural and functional characteristics closely. An AFP from *Colwellia* sp. strain SLW05, an Antarctic sea ice bacterium, was shown to consist of a similar sequence and exhibited ice-binding and ice recrystallization inhibition activities [Raymond *et al*. (2007) *FEMS Microbiol Ecol*, **61**:214-221]. In the present study, we expressed, purified and characterized recombinant *Colwellia* sp. AFP (ColAFP) to investigate its antifreeze and biochemical properties. The recombinant ColAFP exhibited thermal hysteresis of approximately 2ºC at a concentration of 70 μM at a pH of 6.0. The thermal hysteresis activity of ColAFP showed pH dependence and maximized at pH 3-4 (3-fold higher than at pH 6). Since ColAFP and other similar AFPs are secreted to the extracellular space, the pH dependence of these activities seems to be indicative of their physiological adaptation. In the presence of ColAFP, ice crystals "burst" (grow explosively) in a dendritic pattern with hexagonal symmetry at the end of the thermal hysteresis gap, suggesting ColAFP could prevent ice growth along the *c*-axis. By examining the binding capacity of fluorescently-labeled ColAFP to single-ice-crystal hemispheres, we observed that this AFP binds to multiple planes of ice crystal including the basal plane. This is consistent with the finding that affinity for the basal plane of ice is a key determinant of hyperactivity. Interestingly, heat treatment of ColAFP showed no significant decrease of TH activity, even after heating to 100ºC for 5 min. In ColAFP there is a pair of cysteine residues that can form an intramolecular disulfide bond. No cysteine is found in any other related AFP. A disulfide bond in ColAFP presumably contributes to its thermostability. ColAFP might be a unique type of hyperactive AFP characterized by an extremely high thermal stability.

This work was supported, in part, by the Grant-in-Aid for Scientific Research (C) from the Japan Society for the Promotion of Science (JSPS) (No. 20570118) to H.K., and by the CIHR Summer Program in Japan to C.G.