低温適応微生物由来不凍タンパク質の発現系構築と不凍機能の解析

花田祐一 [1,2]、近藤英昌 [2,3]、肖 楠 [1,2]、西宮佳志 [2]、星野 保 [2,3]、三浦 愛 [2]、津田 栄 [2,3]
[1] 北海道大学大学院理学学院、[2] 産業技術総合研究所生物プロセス研究部門、[3] 北海道大学大学院生命科学学院

Expression and characterization of recombinant antifreeze proteins from cold-adapted microorganisms

Yuichi Hanada[1,2], Hidemasa Kondo[2,3] , Nan Xiao[1,2], Yoshiyuki Nishimiya[2], Tamotsu Hoshino[2,3], Ai Miura[2], Sakae Tsuda[2,3]
[1]*Graduate School of Science, Hokkaido University,* [2]*Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology (AIST),* [3]*Graduate School of Life Science, Hokkaido University*

[Background]

Antifreeze proteins (AFPs) have unique functions to bind to ice crystals specifically and depress the freezing point of the solution noncolligatively without affecting the melting point (thermal hysteresis). In AFP solution, the shape of ice crystals are retained within thermal hysteresis range. Ice crystals are grown rapidly once the solution is cooled below the freezing point. Various types of AFPs have been found in a variety of cold-adapted organisms including fish, insects, plants, bacteria, and fungi, showing wide diversities of their molecular sizes, amino acid sequences and three-dimentional structures. Previously, we identified AFP from snow mold fungus, *Typhula ishikariensis*, that shows no sequence similarity to any known AFPs. Recently, it has been reported that Antarctic sea ice diatoms produce AFPs, which share close similarity with *T. ishikariensis* AFP. It has been also reported that fungal AFP-related proteins were found in Antarctic sea ice bacteria, sea ice copepods, and other fungi. It has been proposed that these homologous AFPs would been distributed among the different taxa by horizontal gene transfer. In order to gain experimental insight into the molecular evolution of microbial AFPs, we expressed recombinant AFPs from three different microorganisms (*T. ishikariensis*, basidiomycete; *Navicula glaciei*, sea ice diatom; *Colwellia* sp. strain SLW05, sea ice bacterium) and characterized their antifreeze acitivities.

[Methods]

We utilized the secretion vector pPICZα to express each recombinant AFP in yeast *Pichia pastoris* strain X-33. It was found that the yield of the recombinant AFPs was significantly increased by the induction of AFP expression at a lower temperature. The recombinant AFPs were purified from yeast culture by a specific method based on their affinity for ice. Ice crystal morphology and thermal hysteresis of each AFP solution were observed by a microsope equipped with temperature controller and CCD camera.

[Results and Discussion]

For each AFP, thermal hysteresis exhibited hyperbolic relationship with the protein concentration (Fig.1). *Colwellia* sp. AFP displayed the highest thermal hysteresis value of around 2ºC at a low AFP concentration (0.07 mM). In contrast, *T. ishikariensis* AFP and *N. glaciei* AFP exhibited thermal hysteresis of 0.43 ºC and 0.68 ºC, respectively, even at higher concentration (>0.2 mM).

It has been suggested that the behavior of ice crystal growth is closely related to ice-binding mechanism of AFPs. *T. ishikariensis* AFP produced ice crystals with the irregular shape that burst into two directions below the freezing point (Fig. 2. A). In sharp contrast, ice crystals formed in the presence of *N. glaciei* AFP and *Colwellia* sp. AFP showed rounded shapes and grew explosively into hexagonal directions (Fig. 2. B and C). Both *N. glaciei* AFP and *Colwellia* sp. AFP, therefore, may bind to the ice crystals in a similar manner, but with difference affinity. It is also suggested that another mechanism should be involved in ice-binding of *T. ishikariensis* AFP.

Measurements of thermal hysteresis at various pH also revealed their obvious differences (Fig. 3). *T. ishikariensis* AFP exhibited a constant activity over a wide pH range (pH 2-10), suggesting that the interaction of *T. ishikariensis* AFP with ice does not depend on electrostatic potential of molecular surface. On the other hand, *N. glaciei* AFP and *Colwellia* sp. AFP showed the pH dependence of antifreeze activity; the thermal hysteresis was enhanced at acidic conditions (pH 3-4) and decreased at pH 2 and basic condition. These results indicate that the electrostatic interaction would be involved in ice-binding of *N. glaciei* AFP and *Colwellia* sp. AFP.

To summarize, we have characterized three kinds of AFPs from microorganisms and found that their ice-binding properties are distinct from one another, despite they have similar amino acid sequences.

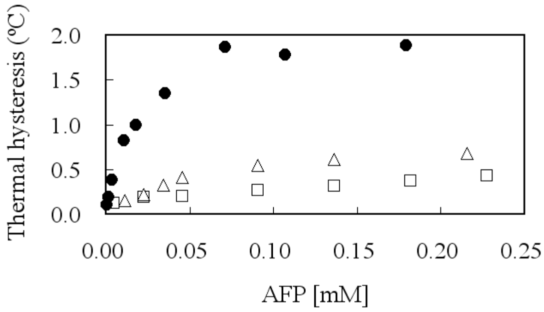

Fig. 1. Thermal hysteresis as a function of the concentration of the AFPs. All measurements were performed in 25 mM MES, pH 6.0. *T. ishikariensis* AFP (square), *N. glaciei* AFP (triangle), *Colwellia* sp. AFP (circle).

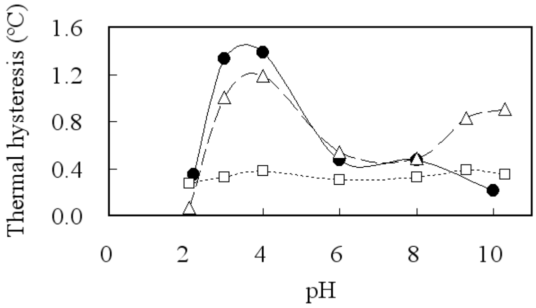

Fig. 3.The effect of pH on thermal hysteresis at the AFP concentration of 0.1 mM (*T. ishikariensis* AFP and *N. glaciei* AFP) and 5 µM (*Colwellia* sp. AFP). *T. ishikariensis* AFP (square, dotted line), *N. glaciei* AFP (triangle, dashed line), *Colwellia* sp. AFP (circle, solid line).

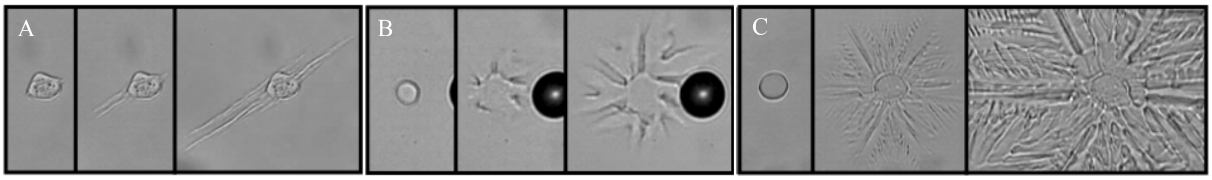

Fig. 2. Ice crystal morphologies and bursts in the presence of *T. ishikariensis* AFP (A), *N. glaciei* AFP (B), and *Colwellia* sp. AFP (C). Microscope photographs of ice crystal above the freezing point (left panel), at the initiation of bursting (middle panel), and on the unrestrained growth of ice crystal (right panel).