Grounds for winning the 2019 Society Award of the Japan Society for Cryobiology and Cryotechnology

Functional analysis of antifreeze protein toward its practical applications

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Dr. Sakae Tsuda received his bachelor's and master's degrees (1985 and 1987) from Hokkaido University (HU) in Japan (JPN). He further continued doctor course student of HU, and quitted it to start working as a teaching profession of high-resolution NMR laboratory operated in HU. He obtained Ph.D. degree (Doctor of Science) in 1992 from HU and moved to Prof. Brian Sykes' lab in U of Alberta (CAN) as a postdoctoral fellow to work on the NMR structural analysis of a muscle protein troponin-C. He returned to JPN in 1994 as an NMR technical advisor of a company (JEOL), and then invited to AIST as a senior researcher in 1995. He obtained the AIST President Award in 2000 for excellence of his work regarding NMR analysis of the structure-function relationship of low-temperature adapted biomolecules. He joined this society in 2003, and has been working as a commissioner from 2009. He organized 2nd International Ice-Binding Protein Conference (IBP2014) at Sapporo in 2014, and also hosted 62th annual meeting of this society at Sapporo in 2017. He has joined editorial board member of several international journals including Scientific Reports. He is now a chief senior researcher of AIST and a professor of HU with 3 staff members and approximately 10 MC & DC students to work on the structure and functional analysis of antifreeze proteins (AFPs).

Dr. Sakae Tsuda explored antifreeze proteins (AFPs) from Japanese organisms, such as, fishes, plants, insects, fungi, and bacteria. He has been clarified DNA sequence, amino acid sequence, biochemical property, ice-binding function, 2ndary and tertiary structure, cell-preservation ability, and structure-function relationship of each AFP species very carefully. He discovered that numbers of fishes living in Japan, some of which are easily available in general food markets, contain type I–III AFPs (AFP I–III) and antifreeze glycoprotein (AFGP). Such discovery first demonstrated that AFPs and AFGP are not special proteins for the organisms in polar area, but are widely distributed in animals and plants living in the middle latitudes that cover Asian countries. He also developed a novel mass-preparation technique of type I–III AFPs and antifreeze glycoprotein (AFGP) by utilizing muscle of Japanese fishes for the first time, and succeeded their commercialization in 2016 as the consequence of collaboration studies for many years with a Japanese company. More details are described below.

(1) Structural and functional analysis of AFP identified from Japanese organisms

He demonstrated that primary sequence and functional property of AFP I–III contained in Japanese fishes are very unique, and are different from those previously isolated from animals living in north America. For example, the AFP I from Barfin plaice consists of an unusually high content of the hydrophobic alanine, though it exhibited an extremely high water solubility (~600 mg/mL). The AFP II from Longsnout poacher has antifreeze activity without Ca^{2+} by a unique ability to expand its target area to whole ice crystal planes with increasing the concentration. The AFP III from notched-fin eelpout consists of 13 isoforms categorized into 6 SP-sepharose binding isoforms and 7 QAE-sepharose binding isoforms, although the corresponding AFP from ocean pout has only 1 QAE-isoform. He synthesized many activity-modified mutants of this AFP III to determine their crystal structures, and discovered that the strongest mutant accompanies “ice-like” water network on its ice-binding site. An observation that several waters in this network showed complementary position match with the waters constructing a single ice crystal allowed him to hypothesize an ice-binding model of AFP III that utilizes the ice-like water network. He determined both NMR and X-ray structures of Japanese original AFPs to deposit to Protein Data Bank (PDB), which includes first crystal structure of a fungal AFP isolated from *Typhula ishikariensis*. On the basis of such structural biologic approach, he further clarified the evolutional origin of sculpin AFPs, the existence of horizontal gene transfer between bacteria and fungi, and the ability of AFP to bind to lipid bilayer that prolongs the life time of a cell.

(2) Development of mass-preparation method of AFP for its application

An ice block that we generally see consists of infinitive numbers of tiny single ice crystals created in water at the moment of freezing. The crystals grow and merge together to form an ice block, if we keep the temperature below 0°C. Type I–III AFPs and AFGP accumulate on the surfaces of such embryonic ice crystals to inhibit their growth and merging, resulting an aggregate formation of the ice crystals instead of an ice block. Dr. Sakae Tsuda noticed that this AFP function is useful for preservation of a variety of water-containing materials such as processed foods, soups, ice creams, noodles, breads, vegetables, seeds, drinks, alcohol, medicines, cosmetics, gels, cells, tissues, and organs. If AFPs can keep the size of each ice crystal to minimum, they may greatly improve the effectiveness of preservation. In addition, fish-derived AFPs bind to the lipid bilayer to prolong the lifetime of cells under hypothermic condition (+4°C), which should be applicable to short-term cell preservation or “cell pausing”. Each AFP and AFGP sample is always a mixture of 2–13 isoforms, which function together far more effectively than any single isoform. He has therefore been trying to develop mass-preparation method of quality products of fish type I–III AFPs and AFGP, and examined their applicability in both industrial and medical fields. He offered these AFP samples to numbers of universities and institutes for free, and made significant contributions to advance both basic studies and applications of a variety of researchers. A unique example of his developed AFP technique is the fabrication of highly porous material using “gelation & freezing method”. Another example is the AFP-containing cell-preservation fluid, which actually prolonged the life time of a bovine cell for 10 days at 4°C to give birth to a healthy calf. From now on, the quality products of AFP and AFGP developed by Tsuda will realize many more advanced techniques.

All of these results show that Dr. Sakae Tsuda made a remarkable contribution to our society through his original activities on cryobiology and cryotechnology. Therefore, we determined that he deserves 2019 Society Award of the Japan Society for Cryobiology and Cryotechnology.