Nature's Ice Fighters: The Antifreeze Proteins and Their Applications

Lavanya M. N.*[1]., Sankalpa K. B.[2]., Sukthija M. P.[3]., Swati S. M.[4]

DSLD College of Horticultural Engineering and Food Technology, Devihosur-Haveri. University of Horticultural Sciences, Bagalkot, Karnataka

Corresponding Author: lavanya.mn@uhsbagalkot.edu.in

Abstract

Antifreeze proteins (AFPs) are specialized proteins known as ice-binding proteins synthesized by wide range of cold adapted organisms, including fish, insects, plants, fungi and bacteria enabling them to survive in cold environments. They will bind to the surface of ice surface to prevents crystal growth by lowering the freeze point without altering the melting point, also exhibits recrystallization of ice, protects cells and tissues. This article provides overview of antifreeze proteins origin, classification, mechanism of action and their applications. AFPs have wide applications in food preservation, frozen foods, cryopreservation, agriculture, and cosmetics. Advanced biotechnology has enabled use of recombinant protein and engineering in AFPs large scale production. Their unique functionality and expanding applications position AFPs as an important innovation at the intersection of biotechnology, food science, and healthcare, with significant potential to address future challenges in sustainable preservation and climate resilience.

Introduction

When temperatures plunge below freezing, life slows down. Water turns to ice, cells rupture, and most living organisms enter a life-threatening state. But not all. Deep in the icy oceans and snow-covered lands, some fish, insects, plants, and microorganisms have evolved a remarkable survival tool called antifreeze proteins (AFPs). These proteins prevents ice growth by attaching to the ice crystals, allowing the organisms to survive in freezing environments without damage. They occur naturally in certain fish, insects, plants, fungi, and microorganisms, acting as a natural defense against freezing (Muñoz *et al.* 2017). AFPs are now being studied for a range of fascinating and impactful applications across industries such as food technology, medicine, agriculture, cosmetics, and more (Xiao *et al.* 2024).

Antifreeze proteins (AFPs)

Antifreeze proteins ae polypeptide compounds that having affinity for ice, synthesised by various organisms such as fish, plants, or insects to survive under sub-zero environment without freezing. In 1969, DeVries discovered AFPs for the first time in the blood and tissues of Antarctic and some northern marine fish, these proteins help to lower the freezing point of fish's blood, without significant increase in osmotic pressure of the plasma, so that the organisms can survive at freezing environment (Ustun & Turhan, 2015 and Davies *et al.* 2013). Currently, AFPs can be found not only in marine fishes but also in snow molds, fungi, sea ice diatoms, snow alga and bacteria (Muñoz *et al.* 2017).

Antifreeze proteins (AFPs) exhibits a unique ability: as they bind to the ice crystals and avoids ice growth, even at sub-zero temperatures. Unlike most solutes that are simply excluded as ice forms, AFPs adsorb directly onto the ice surface, altering the physics of ice formation. A solution containing water and solutes, cooled just below its equilibrium freezing point, ice begins to form. Most solutes including proteins like bovine serum albumin or myoglobin are excluded from the ice lattice and get pushed ahead of the advancing ice front. However, AFPs binds irreversibly to specific sites on the ice crystal surface (Raymond & DeVries, 1977). This blocks further crystal growth in those areas. The only places left for growth are the gaps between bound AFPs, which leads to curved ice surfaces at the microscopic level. This local curvature is critical. According to the Gibbs–Thomson effect, curved surfaces have different thermodynamic properties: it's less favourable for water molecules to attach and extend the crystal in these areas (Wilson, 1994). Thus, further growth of ice is thermodynamically suppressed, unless the temperature is lowered even more. AFPs will help to retain same sizeof the crystal for hours or even days between colligative freezing point (melting point) and the lower non-equilibrium freezing point. The temperature difference between these referred as equilibrium melting point and ice growth temperature is called as thermal hysteresis (TH) (Harding *et al.* 2003).

Classification of AFPs

Based on protein structure and composition of amino acids AFPs are classified into five different types, such as AFPs I-IV type and antifreeze glycoproteins (AFGPs) in fish and two types in insects as shown in table 1.

Table 1: Classification and characterization of AFPs (Uatun & Turhan, 2015; Tejo et al. 2020)

Classification of AFPs	Characteristics
1. Fish AFPs	
Type I	• Simple AFPs, alanine rich α-helix • Has 37 residues, out of which 23 are alanine • Molecular mass ranging from 3.3 to 4.5 kDa • Sources: Right-eyed flounders, Sculpins

Type II	• Largest non-glycoprotein THPs • Protein structure is disulphide bonds between cysteines • 3-dimenional structure with α-helices and two β sheets • Molecular mass ranging from 11 to 24 kDa • Sources: sea raven, smelt and Atlantic herring
Type III	• Sources: Northern and Antarctic eelpout and wolf fish • Molecular mass ranging from 6.5 to 14 kDa • Having two groups i.e. sulfopropyl (SP) and quaternary amines (QAEs) based on their affinity • These AFPs don't have amino acid bias and no cysteine residues • These AFPs are globular or triangular prism with two relatively flat faces and one face of the prism is to bind the ice
Type IV	• Sources: Longhorn sculpin plasma • It is glutamine and glutamate rich protein with 108 amino acids • Molecular mass is approximately 12 kDa • These are alanine rich helical bundles
2. Antifreeze glycoproteins (AFGPs)	• These are having Alanine (Ala), Alanine (Ala), and Threonine (Thr) repeated units • Molecular weight is ranging from 2.7 to 32 kDa, the broad range can be subdivided as • 20-33 kDa- AFGP 1-4 • > 20 kDa- AFGP 5-8 • Sources: Antarctic Notothenioids, northern cods
3. Insect AFPs	• Sources: Beetles (*Coleoptera*) *Tenebrio molitor, Dicentra canadensis, Microdera punctipennis, Anatolica polita, Rhagium inquisitor and R. mordax;* Terrestrial arthropods (spiders, mites and insects) • Some of this AFPs are rich in cysteine, and others are in serine or threonine • These are 10-100 times active than fish AFPs
4. Plant AFPs	• Threonine will be less in plant AFPs, however it shows stronger ice recrystallization inhibition activity • They found in plants which grow in lower temperature environment and can be extracted from any parts of the plant like seeds, stems, crowns, barks, branches, buds, petioles, leaf blades, flowers, berries, roots and tubers

Mechanism of antifreeze proteins (AFPs)

Water crystallizes in two steps: nucleation and propagation of ice crystals. Recrystallization of ice will occur when temperature reaches to sub-zero range through disappearance of small ice crystals and growing to a large ice crystal (Hassas-Roudsari and Goff 2012). The AFPs has properties like depressing freezing point without affecting melting temperature (*i.e.* TH), modifying morphology of ice crystal, inhibiting recrystallization, improving cellular integrity and reducing microbial growth (Ustun & Turhan, 2015). Antifreeze proteins binds to the ice surface and restricts its further growth and in uncovered surface ice growth continuous. This mechanism leads to curvature of the ice crystal by increasing the surface area to volume ratio, makes it thermodynamically unfit for ice growth by non-colligative mechanism and minimizing the osmotic pressure in fish plasma (Jin *et al.* 2026; Kristiansen *et al.* 2005). This unfavourable mechanism causes reducing nonequilibrium freezing point without changing melting point, and known as kelvin effect (Bouvet and Ben 2003).

Sönnichsen *et al.* (1996), explained driving force behind ice binding is manily due to hydrophobic effect. The hydrophobic effect is stronger in Type III AFPs compared with other types and levels of TH activity is strong in insect

AFPs than other AFPs (LI *et al.* 2005). This hydrophobic theory explains interaction of AFPs with ice takes place with total surface area of the protein molecules and the surface is planar, does not have any interruptions, which makes the most hydrophobic AFP surface. The two forces in this model are van der waals forces and adsorption capabilities, also hydrogen bonds involve in binding mechanism (Sönnichsen et al. 1996 & Nutt at al. 2008).

Applications

AFPs has wide application in medical and food industries, where low temperature storage is required or to control recrystallization or to avoid ice growth. AFPs can added to many foods, such as meat and sea foods, fruits and vegetables, flours and dairy products. AFPs can add either directly or immerse in AFPs solutions assisted by ultrasound, vacuum and other methods for proper penetration and also to minimize physical and organoleptic changes (Xiang et al., 2020 & Eskandari et al., 2020).

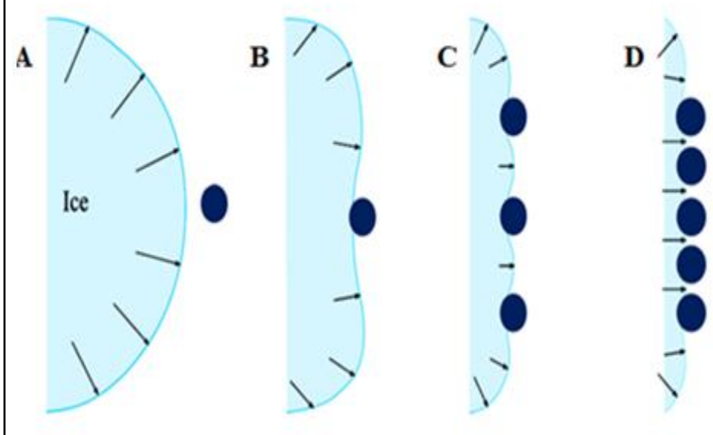

Fig. 1: Schematic representation of Adsorption inhibition Effect of Antifreeze Proteins (AFPs) on Ice Crystal Growth (Tejo et al. 2020).

Table 2: Application of AFPs in food (Xiao et al., 2024)

Application		Source	Concentration	Function
Meat	Chicken breasts	Winter wheat	0.25%(w/v)	The more compact microstructure of myofibril, and smaller ice crystal area and equivalent diameter
	Surimi	Collagen	8%(w/w)	Ca^{2+}atpase activity will be reduced, slow down the oxidation of sulfhydryl group into disulphide bond
	Pork patties	Winter wheat	0.2%(w/w)	Inhibition of water migration and destruction of the microstructure of pork patties during the F-T cycle
	Largemouth bass (Micropterus salmoides)	Herring (Clupea harengus)	0.1% (w/v)	Lowering freezing point and inhibiting recrystallisation
Flour products	Dough and bread	Barley (Hordeum vulgareL.)	0.5% (w/w)	Affect the formation and shape of ice, ice recrystallisation inhibition
	Dough and bread	Carrot (Daucus carota)	0.5%(w/w)	Reduced the increase of frozen water content
	Hydrated gluten	Carrot (Daucus carota)	0.5%(w/w)	Reduced the depolymerisation of glutenin macropolymers (GMP) and weakened the destruction of disulphide bonds, secondary structures, and micro structures
	White salted noodles	Carrot (Daucus carota)	1.29 mg mL^{-1}	Increased cooking absorption and reduced dry matter loss
Fruits/vegetables	Peas	Barley (Hordeum vulgare)	0.9% (w/v)	Ascorbic acid, riboflavin and pyridoxine during cold storage will be reduced

	Green beans	Hippophae rhamnoides	0.1 mg mL^{-1}	Reduced drip loss and electrolyte leakage
	Cucumber	Tenebrio molitor larvae	1 mg mL^{-1}	Effectively maintain the texture of cucumber
	Starfruit (Averrhoa carambola)	Drimys angustifolia	0.1 mg mL^{-1}	Improved drip loss and hardness
	Strawberry	Winter wheat grass	0.2% (w/w)	Improved cell viability and retained the colour of the internal pulp
Dairy products	Ice cream	Winter rye leaves (Secale cereale)	65 mg L^{-1}	Improved the hardness and crystallinity of ice cream and the shape during the melting process
		Porcine skin		Improved the recrystallisation and melting resistance

Summary

Antifreeze proteins are cryoprotectant protein has wide applications in food industries. AFPs interact with ice to arrest ice growth, and recrystallization of ice. AFPs are thermal hysteresis prevents ice recrystallization in variety of organisms without damaging the cells. Apart from biological significance, AFPs have emerged as a valuable biomolecule with diverse application in food preservation, cryomedicine, agriculture, cosmetics, and industrial anti-icing technology. They have ability to maintain quality of freeze food storage, improve cryopreservation of cells and tissues, and enhance frost tolerance. Recent developments in recombinant protein and engineering helps production of AFPs in large scale, which supports adoption of AFPs in industries. Nevertheless, research continues to uncover new resources, mechanisms, application of AFPs in various fields.

Reference

Bouvet, V. and Ben, R.N., 2003. Antifreeze glycoproteins: structure, conformation, and biological applications. *Cell biochemistry and biophysics*, *39*(2), pp.133-144.

Davies, P.L., 2022. Reflections on antifreeze proteins and their evolution. *Biochemistry and Cell Biology*, *100*(4), pp.282-291.

Eskandari, A., Leow, T.C., Rahman, M.B.A. and Oslan, S.N., 2020. Antifreeze proteins and their practical utilization in industry, medicine, and agriculture. *Biomolecules*, *10*(12), p.1649.

Harding, M.M., Anderberg, P.I. and Haymet, A.D.J., 2003. 'Antifreeze'glycoproteins from polar fish. *European journal of biochemistry*, *270*(7), pp.1381-1392.

Hassas-Roudsari, M. and Goff, H.D., 2012. Ice structuring proteins from plants: Mechanism of action and food application. *Food Research International*, *46*(1), pp.425-436.

Jin, Y.C., Liu, Y.T., Gao, X., Yang, X.X., Cao, Y., Ma, Y.Q. and Zhang, H.J., 2026. Source, mechanisms, preparation and potential applications of the antifreeze peptide: a review. *Food & Medicine Homology*.

Kristiansen, E. and Zachariassen, K.E., 2005. The mechanism by which fish antifreeze proteins cause thermal hysteresis. *Cryobiology*, *51*(3), pp.262-280.

LI, J., MA, J. and Zhang, F., 2005. Recent advances in research of antifreeze proteins. *Chinese Journal of biochemistry and molecular biology*, pp.717-722.

Muñoz, P.A., Márquez, S.L., González-Nilo, F.D., Márquez-Miranda, V. and Blamey, J.M., 2017. Structure and application of antifreeze proteins from Antarctic bacteria. *Microbial Cell Factories*, *16*(1), p.138.

Nutt, D.R. and Smith, J.C., 2008. Dual function of the hydration layer around an antifreeze protein revealed by atomistic molecular dynamics simulations. *Journal of the American Chemical Society*, *130*(39), pp.13066-13073.

Raymond, J.A. and DeVries, A.L., 1977. Adsorption inhibition as a mechanism of freezing resistance in polar fishes. *Proceedings of the National Academy of Sciences*, *74*(6), pp.2589-2593.

Sönnichsen, F.D., DeLuca, C.I., Davies, P.L. and Sykes, B.D., 1996. Refined solution structure of type III antifreeze protein: hydrophobic groups may be involved in the energetics of the protein–ice interaction. *Structure*, *4*(11), pp.1325-1337.

Tejo, B.A., Asmawi, A.A. and Rahman, M.B.A., 2020. Antifreeze proteins: characteristics and potential applications. *Makara Journal of Science*, *24*(1), p.8.

Ustun, N.S. and Turhan, S., 2015. Antifreeze proteins: Characteristics, function, mechanism of action, sources and application to foods. *Journal of food processing and preservation*, *39*(6), pp.3189-3197.

Ustun, N.S. and Turhan, S., 2015. Antifreeze proteins: Characteristics, function, mechanism of action, sources and application to foods. *Journal of food processing and preservation*, *39*(6), pp.3189-3197.

Wilson, P.W., 1994. A model for thermal hysteresis utilizing the anisotropic interfacial energy of ice crystals. *Cryobiology*, *31*(4), pp.406-412.

Xiang, H., Yang, X., Ke, L. and Hu, Y., 2020. The properties, biotechnologies, and applications of antifreeze proteins. *International journal of biological macromolecules*, *153*, pp.661-675.

Xiao, S., Hu, S., Zhu, K., Bai, T., Wang, W., Cheng, J. and Zhang, J., 2024. Recent advances, challenges and functional applications of antifreeze protein in food industry. *International Journal of Food Science and Technology*, *59*(3), pp.1169-1186.

* * * * * * * * *