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A Brief Review on Antifreeze Proteins: Structure, Function and Applications.

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ABSTRACT

Antifreeze proteins (AFPs) are novel proteins found in fishes, insects, bacteria and plants in cold climates. They are of varied types but generally follow a similar mechanism of action. 7 general types have been discovered till date depending on the organisms it has been discovered in and its role in preventing ice crystallization. AFPs have a unique property of lowering the freezing point without altering the melting point of ice,aphenomenon known as Thermal Hysteresis. The AFPs work by adsorbing onto the ice surface and restricting the growth of large ice crystals. It prevents ice recrystallization by making the surface thermodynamically unfavourable. CBF/DREB1 proteins of the Arabidopsis family have been found to regulate the expression of cold induced genes. These help improve the tolerance to cold in plants. There are varied applications possible for AFPs ranging from cryopreservation, de-icing, inducing freeze resistance in plants by using genetic manipulation techniques and longer storage in relation to the food industry.

Keywords: Antifreeze proteins, Thermal hysteresis, Ice recrystallization, CBF/DREB1, transgenic plants.

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INTRODUCTION

Antifreeze proteins (AFPs) are a group of proteins that protect organisms living in extremely cold climates from deep freezing temperatures. These are observed in vertebrates, invertebrates, plants, bacteria, and fungi [1].

AFPs do not lower freezing point in proportion to concentration, rather they lower it by working in a non-colligative manner. Allowing them to act at concentrations of 1/300th to 1/500th compared to those of other dissolved solutes. Their presence in low concentrations minimizes the effect of osmotic pressure [2].

AFPs can effectively protect organisms from damage during freezing conditions by a process termed thermal hysteresis(TH).This phenomenon is the presence of a hysteresis gap, that is better explained by the lowering of the freezing point non-colligatively, leaving the melting point unchanged [3].

Freeze tolerant plants avoid freezing by overwintering as seeds with little freezable water; or they can avoid freezing in tissues by super-cooling or lowering the freezing point using antifreeze substances [4]. Generally, the antifreeze proteins bind irreversibly to ice rather than migrating with the ice-water interface. Judged by amino acid compositions, the proteins are found to be relatively hydrophobic. Moreover, this hydrophobicity is accentuated slightly by the clustering of hydrophilic hydrogen-bonding residues to form ice-binding sites. [5].

Antifreeze proteins limit the growth of the ice crystals to manageable sizes without interfering with ice crystal growth hence, they are known as ice-restructuring proteins. When water begins to freeze, many small crystals form and a few of these small crystals dominate and grow larger, utilizing water molecules from the surrounding small crystals. Antifreeze proteins counteract this effect binding to the surface of the small ice crystals and slowing or preventing the growth into larger dangerous crystals [6].

STRUCTURE

Different classes of Antifreeze proteins have been discovered in various plants, fishes and insects, each with a specific characteristic structure but with similar modes of mechanism.

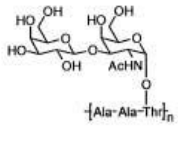
Characteristic	AFGP	Type I AFP	Type II AFP	Type III AFP	Type IV AFP
Mass (Da)	2600 – 33000	3300 – 4500	11000 – 24000	6500	12000
Key Properties	AAT repeat; disaccharide	Alanine-rich α-helix	Disulfide bonded	β-sandwich	Alanine rich; helical bundle
Representative Structure					
Natural Source	Antarctic notothenioids; northern cods	Right-eyed flounders; sculpins	Sea raven; smelt; herring	Ocean pout; wolfish; eel pout	Longhorn sculpin

Figure 1: Structures of antifreeze proteins [7]

There are two categories of fish anti-freeze proteins; the antifreeze glycoproteins (AFGPs) and the anti-freeze proteins (AFPs). The AFGP and AFP make up about 3.4 % of the blood of many Antarctic fishes. In their order of discovery, AFPs are sequentially numbered type I, II, III, and IV [8].

Of the four subtypes, AFP type I is the simplest in structure. It is characterized by its alanine-rich (> 60%) α-helixes and its repeating sequences of 11-residues starting with Threonine[9]. The AFP type I differs from the other AFP's due to its carbohydrate recognition domains (CRDs), which are identical to those of Ca2+ dependant (C-type) lectins. These proteins are around 3.3 - 4.5 kDa [10].

AFP type II is a more complex subtype. It is a globular protein characterized by a high content of disulfide bonds. The type III AFP's are globular proteins with a β-sandwich structure consisting of eight β-strands. It is intermediate in size among the subtypes, with no distinguishing features of secondary structure or amino acid composition.

Type IV AFPs are characterized by high glutamine content (17 %), and its structural fold consists of four α-helix bundles that constitutes about 60% of the total protein. This protein is approximately 12 kDa in size[11].

There are two types of insect antifreeze proteins; namely Tenebrio and Dendroides AFPs. Insect AFPs are more active in inhibiting ice crystal growth than AFPs from fish or plants [12]. Several insect AFPs, sometimes called as thermal hysteresis proteins have been cloned and expressed. They have hydrophilic amino acid residues and are basic in nature. Their maximum activity is 3–4 times that of fish AFPs and are 10–100 times more effective at micromolar concentrations [9]. Throughout the protein length every sixth reside corresponds to cysteine which is important for AFP activity. The 14-20 kDa AFP is a α-helix with a triangular cross-section and rectangular sides that form stacked parallel β-sheets; a fold which is distinct from the three known fish AFP structures. Studies suggest a similarity with a type II fish AFP.

Plant AFPs are found in freeze resistant plants in cold regions. They occur in low concentrations and inhibit the recrystallization of small, extracellular ice crystals into larger, more damaging ones. The AFP of bittersweet nightshade is known to be a 67 kDaGly-rich glycoprotein but its sequence is not fully understood yet [12].

Three types of AFPs have been isolated from winter rye. AFPs from overwintering plants are effective inhibitors of ice recrystallization, possibly due to multiple ice-binding domains that can interact with more than one ice surface. AFP from perennial ryegrass (Loliumperenne) is predicted to fold into a β-rollwith two ice binding domains located on opposite sides of the protein. Alternatively, individual AFPs with single ice binding domains can form oligomers that have multiple ice binding domains, as is the case with winter rye (Secalecereale) [9].

Plant AFPs differ from other antifreeze proteins in the following aspects -they have weaker thermal hysteresis activity compared to other AFPs, their physiological function is likely in inhibiting the recrystallization of ice rather than preventing ice formation and most of them are evolved pathogenesis-related proteins, some showing antifungal properties.

PROPERTIES

Thermal Hysteresis: It results from the lowering of the apparent freezing temperature without actually affecting the melting point. This causes AFPs to be 200 to 300 times more effective at freezing point depressions than in ideal solutions [13].

Inhibition of ice recrystallization: During the recrystallization of ice, larger ice crystals grow at the expense of smaller ones. These are more likely to cause physical damage to tissues and cells. Recrystallization takes place most rapidly at temperatures just below freezing and also when environmental temperatures fluctuate within the subzero range. This effect is seen to be effective at low concentrations of AFPs. The inhibitory effect has been observed in AFGPs, type 1 AFPs from fishes, insect THPs, and plant AFPs [14].

Protective action of ice nucleating agents: Freeze-tolerant insect species produce ice nucleating agents.This significantly increases the crystallization temperature of the hemolymph. It makes the ice crystals to form in the extra cellular spaces at temperatures greater than sub-zero. The ice crystals in the space withdraw water out of solution,resulting in an increase in the osmolarity of the cells since water flows from the intracellular to the extracellular space to compensate for water lost. Thus protecting from intracellular ice formation [15].

Pathogenesis: Most of the antifreeze proteins sequenced has shown that they are homologous to pathogenesis-related (PR) proteins. They are released into the apoplast in response to a pathogenic infection. PR proteins with antifreeze activites have been isolated from winter rye, bittersweet nightshade

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(Solanumdulcamara) and carrot, and include β-1,3-glucanases, chitinases, thaumatin-like proteins and a polygalacturonase inhibitor protein [9].

MECHANISM

The AFPs are found to modify kinetics of freezing by inhibiting the formation and growth of ice crystals. They perform this function by adsorbing on to the ice surface thus restricting the growth of the ice front to regions between the adsorbed protein molecules.Due to this any subsequent growth of an ice crystal occurs on a curved interface, so it becomes less thermodynamically favourable. Adsorbed AFPs can be eliminated from the ice surface by being incorporated into the growing ice crystal. The AFPs show resistance to accumulating ice layers by a certain engulfment angle, which is specific for each AFP [16].

A hydrogen-bonding match was the earliest model proposed to explain binding of AFP to the ice lattice. It was noted that the amphipathic α-helical type I AFP from winter flounder binded to the prism plane of ice through its regularly spaced Thr and Asn residues projecting from one side of the helix [17].

When the solution temperature is further reduced, ice crystal growth occurs primarily on the uncoated, unordered basal plane resulting in bipyramidal-shaped crystals [18].

AFPs get incorporated into the ice lattice by binding irreversibly to the ice surface. The ice binding domains of fish and insect AFPs are flat and relatively hydrophobic.Their adsorption is driven by the increase in entropy gained by the release of hydration water from the ice and protein surfaces. The binding is stabilized by a combination of Van der Waals interactions and hydrogen bonds from hydrophilic amino acids arranged in a strategic manner to match the spacing of the ice lattice [9].

How plants sense the change in temperature is not yet known, although evidence for the involvement of Ca2+ in temperature-sensing and signal transduction has been presented. The regulation of antifreeze activity was investigated in winter rye leaves in response to ethylene, salicylic acid, abscisic acid (ABA) and drought [19]. Non acclimated rye plants treated with salicylic acid and ABA accumulated apoplastic proteins, but showed no antifreeze activity in rye leaves. Non acclimated rye plants exposed to ethylene and drought, showed increase in antifreeze activity and the concentration of apoplastic protein in their leaves [4]. Studies have shown that transcripts and translation products of AFP genes accumulate during cold acclimation. The conditions used for cold acclimation mimic autumn when days are shorter and colder. Thus, low temperature and day length are important environmental cues for AFP production. For example, winter rye plants grown at low temperatures accumulate more apoplastic protein under 8-h days than under 16-h days [20]. The regulation of AFPs is complex because it also involves tissue-specific and developmental responses, as illustrated by studies of cold induction and tissue specificity of AFPs [9].

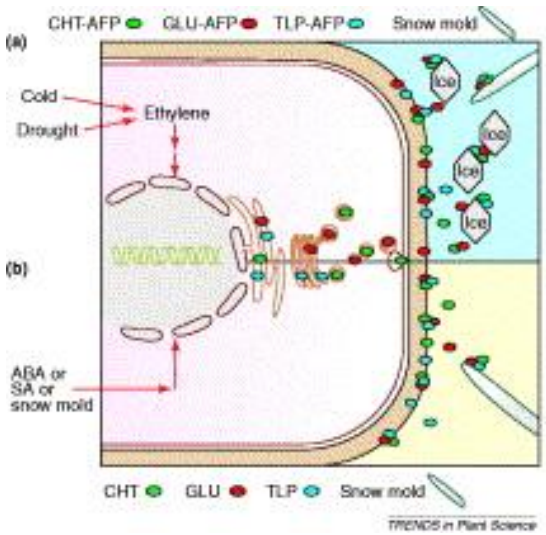

Figure 2: Regulation of antifreeze proteins and pathogenesis related proteins [13].

REGULATION

Regulation is a complex process as it involves tissue specific and developmental responses, like that seen in studies of cold induction and tissue specificity. Additionally a family of Arabidopsis transcription factors, the CBF/DREB1 proteins, are able to regulate the expression of cold-induced genes that increase plant freezing tolerance [21]. How the temperature change is sensed is not known, but evidence for the involvement of Ca2+ in temperature sensing and signal transduction has been discovered [22,4].

APPLICATIONS

In the field of cryopreservation, AFPs are widely used in the cryopreservation of foods that normally are rendered inedible due to ice crystal induced damage. They are also used to cryopreserve tissues and organs [23].

With the rise of global warming and frequent fluctuations in temperature. AFPs are being increasingly used to pioneer the generation of freeze resistant plant species. They can be extracted from organisms, and then injected into the fruits and vegetables resulting in transgenic plants. The offspring of these plants produce antifreeze protein and hence protect from freezing temperature.

AFPs capacity to inhibit ice crystallization, has been sought for maintaining the texture in frozen food, improving storage of blood, tissues and organs, cryosurgery, and protecting crops from freezing[24]. AFP's are being added to the ice cream mixture during the semi-frozen stage, in order to inhibit the ice crystallization and improve the texture of the final product. Doing so, the mixture can be hardened slowly at temperatures from –18 to -30˚C without the formation of large ice crystals. Thus eliminating the necessity for rapid freezing. [25]

CONCLUSIONS

From this paper we conclude that antifreeze proteins play an important role in the survival of plants and animals during overwintering conditions. They have varied mechanisms of action, making it hard to understand their exact nature of activity. A better knowledge of the interaction between structure, function and relationship of antifreeze proteins may allow the design of a more efficient macromolecular antifreeze protein, with a higher thermal hysteresis value per unit concentration. Such an antifreeze protein would be particularly useful for transgenic studies designed to confer improved freeze resistance through gene transfer. The information available on antifreeze proteins in the PDB (Protein Data Bank) is inadequatesince they are large molecules with complex structures, making it hard to sequence them. Incorporating the AFPs into plants sensitive to low temperatures will facilitate their growth in extreme winter conditions, thereby providing a possible solution to the world food crisis situation.

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