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CHARACTERIZATION, STRUCTURE-FUNCTION RELATIONSHIP AND MECHANISM OF ANTIFREEZE PROTEINS

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ABSTRACT

Antifreeze proteins (AFPs) protect organisms from freezing and shows great diversity in structure and they have been found in a variety of organisms including vertebrates, invertebrates, plants, bacteria, and fungi. Study of AFPs is a promising issue due to diverse applications in the fields of industry, medical and agriculture such as food technology, cell lines and organ preservation, transplantation and transfusion. In this review, physicochemical, functional and structural features of some antifreeze proteins of each organism like insects, fish, plant, bacteria and fungi have been described. This review also focused on the structure-function relationship of antifreeze proteins. The result of structure analysis infers that AFPs are mostly hydrophobic and plays the important role in ice binding. In this study, recent new insights into the mechanism of action of four types of AFPs have also been discussed.

Key words: Antifreeze proteins, crystallization, mechanism, structure analysis.

Introduction

The freeze-tolerant organisms are gifted with the AFPs/THPs (antifreeze proteins/thermal hysteresis proteins), which have evolved as an adaptation in extreme cold conditions. THPs have been identified in vertebrates, invertebrates, plants, bacteria, and fungi, and are able to depress the freezing point of water (in presence of ice crystals) in a noncolligative manner by binding to the surface of nascent ice crystals (Gilbert *et al.,* 2004). In many cases, the term *antifreeze* does not imply the natural function of the proteins, as they do not prevent freezing, but rather control the size, shape, and aggregation of ice crystals; hence, the more general term *ice structuring protein (ISP)* has been proposed (Clarke *et al.*, 2002). The THPs comprise a disparate group of proteins with a variety of tertiary structures and often no common sequence similarities or structural motifs. Many high-resolution structures from high field nuclear magnetic resonance (NMR) and x-ray crystallography of AFPs have led to the elucidation of a structure–function relationship (Sonnichsen *et al.*, 1993, 1996; Sicheri and Yang, 1995; Gronwald *et al.*, 1996; Jia *et al.*, 1996; DeLuca *et al.*, 1998). AFPs represent a remarkable example of parallel and convergent evolution, with different proteins being adapted for the antifreeze role (Cheng and Chen, 1999; Ewart *et al.*, 1999). In this review, we deal with the sources, physicochemical, functional and structural properties of some antifreeze proteins which exist in different organisms. Furthermore, the present study focuses the new insights into the mechanism of action of various types of AFPs and AFGPs and the development of methodology to allow the production of synthetic AFPs as well as their potential uses.

DIVERSITY AND DISTRIBUTION OF AFP

Classification of AFPs

AFPs are observed in extracts obtained from organisms from four of the five kingdoms Monera, Fungi, Plantae, and Animalia (Duman and Olsen, 1993; Duman *et al.*, 1993; Griffith and Ewart, 1995; Griffith *et al*., 1997; Ewart *et al.*, 1999; Hoshino *et al.*, 1999; Yu and Griffith, 2001). There are two main types of AFPs: glycoproteins and nonglycoproteins (Hew and Yang, 1992). The AFPs (nonglycoproteins) are classified essentially according to (Davies and Hew 1990) into types I to IV and antifreeze glycoproteins

(AFGPs) (Table 1). The AFGPs have been very effective for many purposes (Harding *et al.*, 2003). The AFGPs constitute the major fraction of protein in the blood serum of Antarctic Notothenioids and arctic cod. Each AFP consists of varying number of repeating units of (Ala-Ala-Thr) with minor sequence variations, and the disaccharide *β*-D-galactosyl-(1→3)-*α*-N-acetyl-D-galactosamine joined as a glycoside to the hydroxyl oxygen of the Thr residues. These compounds allow the fish to survive in subzero temperatures. Studies have shown that AFGPs are largely unstructured in aqueous solution. Although standard carbohydrate degradation studies confirm the requirement of some of the sugar hydroxyls for antifreeze activity, the importance of some structural elements has not been established.

Sub-classification of AFPs

The type I AFPs are of two subtypes, the flounder and sculpin subtypes. The flounder type is built up of repeat of eleven amino acids (TxxNxxxxxxx), where x is principally alanine and N sometimes aspartate or threonine, and the sculpin is non-repetitive and more amphipathic with several lysine and arginine side chains projecting from the same face of the helix (Sicheri and Yang, 1995; Chao *et al.*, 1996b; Davies and Sykes, 1997). The type II AFPs are divided on the basis of Ca^{++}requirement for their activity. They are homologous to the carbohydrate recognition domain of Ca-dependent lectins (Ewart *et al.*, 1992, 1996, 1997; Chao *et al.*, 1995; Sonnichsen *et al.*, 1995). The herring and smelt type II AFPs are Ca^{++}dependent, whereas the sea raven type II AFPs are Ca^{++}independent for their activity. The smelt type II AFP differs from the other two because it contains small amounts of glucosamine (Davies and Sykes, 1997).

Table 1. Properties of antifreeze protein (Davies and Sykes, 1997; Harding *et al.*, 2003)

Characteristic	AFGP	AFP Type I	AFP Type II	AFP Type III	AFP Type IV
Mass (kDa)	2.6–3.3	3.3–4.5	11-24	6.5	12
Primary structure	(AAT) repeat;	A rich	Disulfide stabilized	General	Alanine rich or glutamine/ glutamate
Amino acid bias	>60% A; >30% T	A∼60%	Cysteine rich 9%	General	Glutamine/ glutamate rich (26%)
Secondary structure	Extended 3-fold helix	*α*-Helix	Mixed coil	*β*-sandwich	*α*-Helix
Tertiary structure	Extended	Single *α*-helix	Globular, C-type lectin fold	Globular	Helical bundle
Heterogeneity	Polymer 1–8	Repetitive/non-repetitive	Ca-dependent/ independent	Isoforms, pIs 6–10 dimer	NA
Ice binding	Close to 10–10	20-21/2-1-10	11-21	Close to 10-10	NA
Homologues or antecedents	Trypsinogen gene	NA	Ca-dependent lectins and Lithostathine	NA	Lipoprotein domain
Natural source	Antarctic notothenioids; northern cods	Right-eyed flounders (winter flounder, two yellow tail flounder) Sculpins (Shorthorn Grubby)	Sea raven, smelt, herring	Ocean pout, wolfish, eel pout	Longhorn sculpin
Expression or synthesis	Chemical synthesis or natural source	Recombinant and natural source	Recombinant and natural source	Recombinant and natural source	Natural source

PROPERTIES

Melting Point/Freezing Point Depression

The melting point (MP) or freezing point of a solution is the temperature at which the solid phase disappears when a frozen solution is slowly heated without change in temperature (Zachariassen and Kristiansen, 2000). The AFP reduces the freezing point without affecting the melting point (Davies and Hew, 1990).

Thermal Hysteresis

Super cooling is a phenomenon in which aqueous solutions remain in the liquid state when cooled below the MP; at this state, the system is said to be in metastable equilibrium. The temperature at which spontaneous nucleation occurs in a super cooled solution is termed as the super cooling point (SCP) of the solution. The super cooling capacity is the difference between the MP and the SCP. The difference between the MP and the temperature at which ice growth occurs is termed *thermal hysteresis* (TH), and the temperature at which spontaneous ice growth takes place is referred to as the *hysteresis freezing point* (HFP). The difference between the MP and the HFP is the "hysteresis" or "hysteresis activity" (Zachariassen and Kristiansen, 2000). The AFPs lower the freezing point of water (in the presence of ice crystals) without significantly altering the MP. The observed effect of lowered freezing point is a thousand fold greater than expected for the molar concentration of THP. Hence, the mechanism is non-colligative and will have little effect on osmotic pressure. However, higher concentrations of protein are required to exhibit this activity compared to re-crystallization inhibition. AFPs work additively with colligatively active substituents such as salts and amino acids.The non-colligative activity may be as high as 500 times that of colligatively acting substances (Mishra and Pattnaik, 1999; Zachariassen and Kristiansen, 2000). At a certain concentration of AFP, the activity saturates and a plateau is reached; a further increase in the concentration of AFPs does not result in a further reduction of freezing point (Yeh and Feeney, 1996).

Inhibition of Ice Recrystallization

Ice crystals present in a solution at constant temperature will gradually change their size (i.e., the large crystals grow larger at the cost of smaller ones, which disappear). This phenomenon is referred to as *recrystallization* and is ascribed to the differences in surface curvature (i.e., Kelvin effect) (Griffith and Ewart; 1995; Davies and Sykes, 1997; Zachariassen and Kristiansen, 2000). Recrystallization of ice is more likely to cause physical damage to tissues and cells. Recrystallization takes place most rapidly at temperatures just below freezing point and during warming from the glassy state. Ice also recrystallizes when environmental temperatures fluctuate within the subzero range. Knight *et al.* (1995) reported that at high subzero temperatures there is some liquid around the ice grains, which allows ice recrystallization, resulting in an energetically favorable state in which the total surface area of ice is reduced. Extremely low concentrations (100 μg L–1) of AFP are effective in inhibiting ice recrystallization (Knight *et al.*, 1984, 1988; Yeh *et al.*, 1994). Although it is likely that recrystallization inhibition stems from the interaction of AFPs and ice crystals, the exact mechanism involved in recrystallization inhibition at very lowconcentration of AFPs is not understood.

Interaction with Ice Nucleators

Ice-nucleating proteins (INPs) are those that promote the formation of ice at subzero temperatures by mimicking the structure of the ice crystal surface and acting as seed ice crystals or as a heterogeneous ice nucleus. Ice-nucleating activity (INA) has been subdivided into three types. Type I nucleators, which nucleate water at or above –5◦C, are the most active natural nucleators with the exception of ice itself (Yankofsky*et al*., 1997). Type II nuclei are active between –5◦C and –7◦C, and type III nuclei are active below –7◦C (Cochet and Widehem, 2000). They are produced by at least six bacteria and *Fusarium* and related genera of fungi (Kawahara, 2002). Because AFPs bind to the surface of ice crystals or to the ice nucleus, they probably also bind to INPs by a similar mechanism, inactivating activity, thus preventing further growth of the ice nucleation network (Duman *et al.*, 1991; Mishra and Pattnaik, 1999). Although it is presumed that the effect of AFPs and INPs cancel each other due to their antagonistic activities, the

presence of both these proteins have been reported to exhibit enhanced antifreeze activity, after the formation of AFP-INP complex (Duman *et al.*, 1991). The previous report gains relevance as it has been foundthat*Pseudomonas fluorescens* KUAF-68, isolated from Antarctica, produced both AFPs and INPs (Kawahara *et al.*, 2004).

ANTIFREEZE ACTIVITY ASSAY

TH is measured *in vitro* with the use of a Clifton nanoliter osmometer. Submicroliter volumes of AFP solution are introduced into an oil droplet, which is held by surface tension in a cylindrical well drilled in a metal plate. The whole set-up is then placed on a cooling stage and viewed under a microscope. The sample temperature is controlled by a Peltier device with a read-out in mosmols. Ice crystal morphology and its growth during the TH measurement could be studied by still or video photography though the microscope (Fletcher *et al.*, 2001). Recrystallization inhibition (RI) activity end point has been determined for many AFPs by using this method (Knight *et al.*, 1995; Worrall *et al.*, 1998; Smallwood *et al.*, 1999; Sidebottom *et al.*, 2000; Raymond and Fritsen, 2001). The major problems with these methods are that the samples cannot be stored for future use or analyzed simultaneously. Tomczak *et al.* (2003) devised the capillary method for determining RI activity of AFPs. In this method, serial dilutions were prepared to determine the concentration below which RI activity was no longer detected, termed as*RI end point*. This procedure helps in viewing of samples simultaneously, as well as preserving them for future use.

SOURCE

Insect AFPs

AFPs are reported in many species of insects and in many terrestrial arthropods (Duman *et al.* 2004; Tursman and Duman, 1995; Sjursen and Somme, 2000). The insect AFPs decrease the freezing point of solutions by about 6◦C (Graham *et al.*, 1997; Tomczak *et al.*, 2003). Some of the AFP-producing insects are freeze tolerant and are able to survive very low temperatures (−40◦Cto−70◦C). The antifreeze activity in the presence of insect AFPs is shown to increase with decreasing crystal size (Hansen and Baust, 1988; Duman and Serianni, 2002). In addition to AFPs, body fluids may contain compounds that act as catalysts for the growth of ice, called *ice nucleators*. AFPs from *D. Canadensis* have been shown to inhibit ice nucleator activity (Olsen and Duman, 1997; Duman, 2002). Kristiansen *et al.* (2005) isolated six AFPs from the long horn beetle, *Rhagium inquisitor*. Andorfer and Duman (2000) found that *D. canadensis* expressed some thirteen isoforms. *T. moliter* expressed twelve isoforms (Liou *et al.*, 1999), while *C. fumiferana* expressed 13 (Douchet*et al.*, 2002). AFPs are also isolated from the beetle, *Tenebrio molitor* (Graham *et al.*, 1997), and a moth, *Choristoneurafumiferana* (Tyshenko*et al.*, 1997). The spruce budworm *C. fumiferana* antifreeze proteins (CfAFPs) are divided into three classes based on the length of 3_UTRinto long, intermediate, and short 3_UTR. Liou *et al.* (1999) also isolated a complex family of highly heterogeneous and internally repetitive hyperactive AFPs from the beetle, *Tenebrio molitor*. It has been suggested that the disulphide bonds of insect AFPs also prevent structural unfolding at low temperatures (Graether and Sykes, 2004). The most distinguishing feature between AFPs from fish and those from insects is that the latter has a considerably higher activity (10–100 times greater activity). Fish in polar waters are exposed to temperatures as low as-1.9◦C, whereas over wintering insects can experience temperatures of –20◦C or lower (Fletcher *et al.*, 1998; Ewart *et al.*, 1999).

Fish AFPs

AFPs have been identified in many species of fishes (Brown and Sonnichsen, 2002). The AFPs in the fish winter flounder possess two different antifreeze gene families, one of which is expressed in liver and is secreted out to the blood, and another is expressed in the gills and skin epithelia for protection of the cells and tissues that come into direct contact with external ice (Gong *et al.*, 1996; Fletcher *et al.*, 2001). Four different types of AFPs and AFGPs (a total of five) are reported in fishes (Ewart *et al.*, 1999; Brown and Sonnichsen, 2002). The fish AFPs show a TH of between 0.6◦C and 1.5◦C (Fletcher *et al.*, 1998; Ewart *et al.*, 1999). This low TH range is attributed to the fact that the fish AFPs bind to only one plane, although at higher concentrations they may be able to bind to additional planes (Wilson *et al.*, 2002). Marshall *et al.*

(2004) described a new AFP from the flounder that is as active as those found in insects and which explains the resistance of this fish to freezing in polar and subpolar waters.

Plant AFPs

The plant AFPs thermal hysteresis range is only 0.2◦C to 0.4◦C, but is an extraordinary inhibitor of ice recrystallization. This inhibition differs from freezing point depression in that it requires 100 to 500 times less AFP. In plants, the AFPs are associated with the cell wall, other cell organelles, and the intercellular spaces. This suggests that these proteins may control the propagation of ice crystals in tissues inward from the epiphytic ice nucleators or outward from the vascular bundle. They act by modifying the crystallization of ice propagators throughoutthe plant (Griffith *et al.*, 1997; Hoshino *et al.*, 1999; Maunsbach*et al.*, 2001; Atici and Nalbantoglu, 2003). Over wintering plants produce AFPs that have the ability to adsorb onto the surface of ice crystals and modify their growth (Griffith *et al.*, 1997; Ewart *et al.*, 1999; Hoshino *et al.*, 1999; Yu and Griffith, 2001). Several AFPs have been isolated and characterized, and some cloned from higher plants (Worrall *et al.*, 1998; Meyer *et al.*, 1999; Sidebottom *et al.*, 2000; Yeh *et al.*, 2000). Kontogiorgos*et al.* (2007) isolated a heat-stable protein that belongs to thaumatin family with RI activity. Structural studies using CD showed that the protein consists of ß-sheet and random coil. It is reported that at low temperature (Worrall *et al.*, 1998; Smallwood *et al.*, 1999), the hormones ethylene (Yu *et al.*, 2001), and abscisic acid (Dave and Mitra, 1998; Lu *et al.*, 2000) are involved in regulating antifreeze activity in response to cold. AFPs in winter rye have more than one function. It has been shown that some AFPs are similar to the pathogenesis-related (PR) proteins identified as glucanase-like, chitinase-like, and thaumatin-like proteins (Yeh *et al.*, 2000; Atici and Nalbantoglu, 2003).

Bacterial and Fungal AFPs

Microorganisms not only exist, but also thrive in extremely cold environments such as the Antarctic and Arctic (Kawahara, 2002). The AFPs produced in bacteria have lower TH activity than animals (Duman and Olsen, 1993; Sun *et al.*, 1995; Yamashita *et al.*, 2002). These organisms readily freeze, but use the RI activity of AFP to control the size of ice crystals (Griffith and Yaish, 2004), suggesting that they employ a freeze-tolerant strategy. Most of the reported bacterial AFPs are exported from the cell (Duman and Olsen, 1993; Sun *et al.*, 1995; Yamashita *et al.*, 2002), and localize in the periplasm where they bind and inhibit nucleating ice crystals from the extracellular environment before they damage cells. Gilbert *et al.* (2004) isolated eleven bacteria strains that produce AFPs from Antarctic lakes. In the bacterium *Marino monasprimoryensis*, a highly active AFP has been observed similar to insects and fishes, pointing out that freeze-avoidance strategy does exist in bacteria (Gilbert *et al.*, 2005). Yamashita *et al.* (2002) screened 130 strains and found six bacteria capable of producing AFP from Antarctica. Among these six strains, they identified the highest antifreeze activity from a 52-kDa lipoprotein from *Moraxella* sp. One of the bacterial strain identified as *Pseudomonas fluorescens* produced both AFP and INPs, although both these activities are mutually inhibitory (Parody-Morreale *et al.*, 1988). A similar observationwas also made in *P. putida* (Sun *et al.*, 1995; Kawahara, 2002). Among fungi, *Pleurotus ostreatus* and *Flammulina velupites* and some bracket, such as *Coriolus versicolor*, have been foundto show TH in their cellular extracts (Duman and Olsen, 1993).The psychrophylic basidiomycetes, *Coprinus psychromorbidus*, was reported to produce three kinds of TH proteins (23 kDa)in the extracellular space and are different from each other intheir N-terminal amino acid sequences.(Hoshino *et al.*, 2003).Newsted *et al.* (1994) reported that *Myriosclerotina borealis* and *Typhula incarnate* produced proteins, which were shown tohave antifreeze properties by NMR microimaging experiments.They also reported the production of a small size protein (3,500Da), which has epitopic homology to the Atlantic winter flounderAFPs from snow molds.

MECHANISM OF ICE GROWTH INHIBITION

All types of AFPs differ in their structure and amino acid composition, yet they act in a remarkably similar way. When the super cooling temperature of solutions is slightly more than the level of freezing temperature suppression, the growth morphology of ice crystals is very different from that in water in presence of AFPs (Griffith *et al.*, 2005). Recrystallization of frozen solutions is inhibited at very low

concentrations (<0.1 μg/mL) (Knight *et al.*, 1984, 1988). A possible mechanistic explanation for THP activity is given by the Gibbs-Thomson model (Yeh and Feeney, 1996).

Raymond and DeVries (1977) proposed the adsorption-inhibition hypothesis. The AFPs are adsorbed on the ice crystal surface or possibly by some type of interaction at the ice–water interface (Chapsky and Rubinsky, 1997; Grandum*et al.*, 1999).Wen and Laursen (1993) proposed a two-step adsorption and growth inhibition mechanism. In their model, patches or aggregates of AFP molecules bind tightly to the ice surface. Kristiansen and Zachariassen (2005) explained TH in terms of vapor pressure equilibrium prior to a surface nucleation event and ascribed the antifreeze effect to the pressure build-up within the convex structure. Initial evidence for adsorption of AFP onto ice crystals has been gathered from three type of analysis. First, infrared spectroscopic measurements of solutions containing ice and AFP generate second harmonics at the ice–water interface, which is not observable in pure ice–water interfaces. Second, unlike most solutes, AFPs appear to be incorporated into growing ice crystals during freezing (Raymond and DeVries, 1977; Knight *et al*, 1991, 1993). Third, the transition temperature during freezing is sudden for the AFP solution, in contrast to more gradual transition for solutions in which solute is excluded from ice (Raymond and DeVries, 1977).

Sicheri and Yang (1995) resolved the crystallographic structure of type I AFP. The type I AFP has N- and C-cap structures that help explain the ability of the protein to maintain its helicity. It is shown that for type I AFPs, the helicity is an important factor, and substitution of A20P produces an inactive variant. The conserved Ala-rich face has been shown to be important for ice binding, and residues with long side chains, if substituted for Ala, can sterically prevent AFP binding ice. The Thrγ-methyl group has also been shown to be essential from the retention of activity in Thr to Val substituents (Jason and Davies, 2002). These results support a mechanism of action based on hydrophobic and van der Waals effect. Zhang and Laursen (1999) synthesized α-helical peptides sequences having positive charged and nonpolar side chains, which are analogous to those of type I AFPs. It was found that lysine residues, when properly positioned on a α-helical polyalanine scaffold, exhibited antifreeze activity. Grandum*et al.* (1999) studied the growth pattern of ice related to the potential for crystal growth as well as the crysl surface topography. Inglis *et al.* (2006) used pulsed field gradient spin echo NMR spectroscopy to measure the diffusion coefficient of type I AFP from winter flounder and also for the synthetic derivatives of AFPs. These synthetic derivatives of AFPs represent changed amino acid profiles with that of the native AFPs, wherein the four Thr residues of the native type I AFPs were replaced with either Val or Ala. Liu and Li (2006) derived a model for the binding of AFPI to a two-dimensional ice lattice based on the ligand site size and intrinsic binding constant. They showed that the difference in antifreeze activities of these proteins stems from ice surface coverage, in addition to affinity and specificity for ice surface. Analysis of primary sequences of the AFPs revealed that residues at some positions are highly conserved. Mutational studies have been successfully used to elucidate the ice binding face of these proteins. Steric mutations were used to identify the ice binding face of type I, II, and III AFPs (Cho *et al.*, 1994; Jason and Davies, 2002).

Many high-resolution structures from high-field NMR and x-ray crystallography has been conducted on type III AFPs (Sonnichsen *et al.*, 1993, 1996; Jia *et al.*, 1996; DeLuca *et al.*, 1998; Ye *et al.*, 1998). The protein exhibited a compact fold, with a relatively large hydrophobic core, and several short and irregular β-sheets with one helical turn. The ice binding site was constituted by parts of C-terminal sheet and a loop, is planar and relatively nonpolar, and has low solvent accessibilities and the specific spatial arrangement of polar side chain atoms of putative ice binding regions. Jason and Davies (2002) investigated the contributions of hydrophobic interactions between type III AFP and ice by generating single and double mutants. They showed that side chain substitutions, which leave a cavity or undercut the contact surface, are as deleterious to activity as those of lengthened chains, emphasizing the importance of surface contour on ice binding face for docking to ice. Madura *et al.* (1996) conducted molecular dynamic simulation studies on type IIIAFPinwater, for elucidatingprotein–water interactions, and compared with NMR structure in order to test the proposed ice binding surface by Cho *et al.* (1994). They also explored its interaction with ice planes using an averages simulation structure. Dynamic simulation studies revealed

AFP to be heterogeneous in three-dimensional as well as amino acid sequences with a wide distribution of polar and charged residues in agreement with the NMR study. Gallagher and Sharp (2003) investigated the hydration structure of type III THP and found significant differences in the hydration structure of the ice binding face compared to non– ice binding protein surface and non-THP of similar size and structure. Yang and Sharp (2004) showed that a more ice-like hydrating water structure was formed on the ice binding faces of active THPs, implying that this protein has a high affinity and specificity for more ordered or ice-like water or ice itself. The structure–function relationship suggests that this affinity is due to a dual characteristic of the ice binding surface. Some authors have shown contributions of hydrophobic residues on the periphery of the putative ice binding site for TH activity (Chen and Jia, 1999; Baardsnes and Davis, 2002).

Li *et al.* (1998) studied the secondary structure of *Dendroides*AFP, using infrared and CD spectroscopies, and revealed thatthe eight disulfide bridges imposed significant constraints onpotential secondary structural features. Graether*et al.* (1999) crystallized AFP from spruce budworm by the hangdrop method. Liou *et al.* (2000b) observed a good two-dimensional match to ice lattice and showed that all three ranks of oxygen atoms (two from threonine and one from water) can be achieved with a 0.5 deviation, which implies that ordered water molecules could act as part of the ice binding surface and could participate in AFP–ice interactions. Daley *et al.* (2002) studied that the rigid and hydrophobic binding site might reduce the entropic penalty during ice binding. Wathen *et al.* (2003) developed computational techniques for modeling crystal formation. Results of modeling the inhibitory effect exerted by fish and insect AFP on ice crystal formation were consistent with what was observed experimentally. Scotter *et al.* (2006) compared the activity of hyperactive AFPs with that of moderately active AFPs by cooling below the non-equilibrium freezing temperature. Mixtures of different AFPs produced ice crystals of hybrid shapes and dimensions, consistent with the fact that different antifreeze types binding to the same ice surfaces are also reported (Ben, 2001; Hamada *et al.*, 2006). The antifreeze activity was also found to be independent of the proportions of isoactive AFP stocks, indicating that different AFPs neither attenuated nor potentated each other's activity. Thus, it may be said that AFP molecules are independently active and do not require protein– protein interactions for ice binding.

Observations of the review study

Antifreeze proteins have the ability to reduce freeze temperature and can be found in bacteria, fungus, insects, plants animals etc. AFPs can be various types on the basis of their functional characters. We have observed the different properties, functional characteristics as well as mechanisms.

Conclusion

Antifreeze proteins (AFPs) are a group of proteins that protect organisms from deep freezing temperatures and are expressed in vertebrates, invertebrates, plants, bacteria, and fungi. All types of AFPs differ in their structure and amino acid composition. Most of the AFPs known are extracellular and hydrophobic in nature. They play an important role in ice binding that can be used for cryopreservation, food technology as well as in agriculture.

Uses of the study

The different sources, types and functional properties of AFPs make them promising for the versatile use in preservation, food industry, plants and animal.

Recommendations from the study

The further study may be performed on the specific uses, production technology for commercial scale and computational identification of AFPs for generating transgenic organisms.

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