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POTENTIAL USES OF ANTIFREEZE PROTEINS: A REVIEW

A. Mimi[1], M. R. Amin[1], S. M. Ahmed[2], M. A. Haque[3] and M. Z. Tareq[1]
[1]Genome Research Centre, BJRI, Dhaka-1207
[2]CI Molecular Genetics, ASRBC, ACI Agri-business, ACI Limited, Gulshan, Dhaka
[3]Dept. of Biotechnology and Genetic Engineering, Islamic University, Kushtia-7003

ABSTRACT

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. AFPs have been identified in vertebrates, invertebrates, plants, bacteria, and fungi. In this review, we deal with, diverse applications in the fields of industry, medical and agriculture such as food technology, cell lines and organ preservation, transplantation and transfusion. AFPs have potential applications in agriculture for the production of economically valuable fishes against low temperature. Other proposed applications of AFPs are found in cryosurgery of tumors and as a component of ice-cream to prevent the formation of hard and large ice crystals. Furthermore, the present study focuses the new insights into the development of methodology to allow the production of synthetic AFPs as well as their potential uses.

Key words: Antifreeze protein, cryopreservation, food safety, cryosurgery.

Introduction

Antifreeze proteins (AFPs) were first detected in Arctic fish (Scholander *et al*. 1957) and later grouped into types I, II, III and IV based on their sequences and structures. AFPs discovered in microorganisms such as bacteria and fungi (Gilbert *et al*.2005; Singh *et al*. 2014). Similarly, AFPs have been observed in plant (Gupta and Deswal 2014), in insects (Patterson *et al*. 1981; Duman *et al*. 2004). Antifreeze proteins have been isolated from many different organs, such as the liver, stomach, heart, seeds, stems,bark, leaves, and flowers (Cheung *et al*. 2017). AFPs are highly sought after for use in cryopreservation, biotechnology, and the food industry (Christner 2010) owing to their unique abilities. The addition of AFPs to cells, organs,and tissues of plants and animals has been shown to improve cryopreservation efficiency (Jeon *et al*. 2015; Seo *et al*. 2018).With regards to food, AFPs improve the texture of ice cream (Regand and Goff 2006) and the quality of preserved meat (Griffith and Ewart 1995). This review discusses the applications of diverse AFPsin food, cryopreservation as well as plant and animal biotechnology in detail.

BIOLOGICAL FUNCTIONS AND APPLICATIONS OF AFPs

AFPs act as protecting agents in cryogenic or hypothermic storage of whole organisms in isolated organs, such as liver, tissue or cell lines, oocyte, sperm, embryos, red blood cells, and food preservation (Griffith and Ewart, 1995; Feeney and Yeh, 1998). A US patent for this beneficial effect has also been filed (Rubinsky *et al.*, 1994a). AFGPs could prevent leakage from liposomes composed solely of phospholipids as they are cooled through their phase transition temperature (Hays *et al.*, 1996). This suggests that the peptides interact with lipids to stabilize membranes at low temperature. On the contrary, a physiological mixture of AFGP, AFP I, and AFP III are cytotoxic to plant thylakoids, as determined by the leakage of plastocyanin, a soluble thylakoid lumen protein. In contrast, the smallest fraction of AFGP offers a limited degree of protection during freezing and does not induce fusion, which is dependent on concentration (Tomczak *et al.*, 2001).

Food Technology

AFPs may inhibit recrystallization during freezing storage, transport, and thawing, thus preserving food texture by reducing cellular damage and also minimizing the loss of nutrients by reducing drip (Griffith and

Ewart, 1995; Breton *et al.*, 2000). It is well known that some food items, such as fruit and vegetables (e.g., strawberries, raspberries, tomatoes), cannot be frozen without loss of quality caused by cellular destruction. Fletcher *et al.* (1997) patented a technique for the expression of AFP in lactic acid bacteria during the fermentation process for the production of yogurt. The transgenic bacterium can be used for sauerkraut, pickles, and miso, and thus avoids the necessity of purifying AFP prior to addition to food. The cold induced winter rye enzymes, such glucanase and chitinase, exhibit both antifreeze and enzymatic activity (Hon *et al.*, 1994). Mueller *et al.* (1991) demonstrated the inhibition of ice recrystallization by chimeric proteins containing an antifreeze domain. Thus, AFPs could be engineered with other proteins bearing specific functions, which will help augment the quality of food.

Safety Aspects for Introduction into Food

Food allergy, defined as an IgE-mediated reaction, is estimated to affect 1% to 2% of the total population and 5% to 8% of young children in the industrialized world (European Commission, 1998). Crevel*et al.* (2002) reviewed the occurrence of AFP and related it to the likely intake by humans, with a view to judge their safety in food. Metcalfe *et al.* (1996) devised a decision-making tree based on allergic parameters, which has been adopted by Food and Agricultural Association of the United Nations/World Health Organization (FAO/WHO) (WHO, 2000, 2001). Manning *et al*. (2004) studied that AFP did not exhibit genotoxic potential or sub chronic toxicity during oral administration in rats. Crevel *et al*. (2007) also showed that ISP does not have any effect on general health nor is it immunogenic in lieu of other studies.

Cryopreservation

Cryopreservation of cells, tissues, and embryos is one of the most promising areas, with immense applications to both medical and biotech industries. The survival of cells and tissues depends on the rate of cooling and warming, type and concentration of cryoprotectant and the type of cells used. Evidence suggests that AFPs from winter flounder exhibit the ability to block ion channels, specifically suppressing Ca++ and K+ fluxes across cell membranes (Rubinsky *et al*., 1992). Fish AFPs have been found to improve the cryopreservation of oyster (Crassostreagigas) oocytes (Naidenko, 1997), carp sperm (Karanova *et al*., 1997), bovine and porcine oocytes (Rubinsky *et al*., 1991), red blood cells (Chao *et al*., 1996a) and vertebrate and invertebrate cell lines (Koushafar and Rubinsky, 1997), intact livers (Lee *et al*., 1992) as well as embryos (Baguisi *et al*. 1997). Prathalingam *et al*. (2006) suggested that bull spermatozoa cryopreserved in the presence of AFPI may have increased fertility in vivo. Robles *et al*. (2006) injected AFPI into the embryos from Sparusaurataat different stages and subjected them to two different temperatures 0◦C and –10◦C. AFPI was shown to increase the resistance to chilling and displayed nearly 100% hatching rates. However, Ishiguo and Rubinsky (1998) showed that the presence of AFP changed the morphology of ice crystals and resulted in the complete destruction of RBCs. From the forgoing, it may be concluded that the application of AFP technology in cell preservation requires experimental validation, as the effect is dependent on the type and concentration of AFP used as well as the composition of membrane milieu of the cell.

State of Proteins during Freezing and Thawing

Proteins are routinely stored as frozen solutions in research laboratories, with the anticipation that the long-term stability of the frozen state will be greater than that noted in unfrozen solutions. Freezing-induced damage to proteins is also a major stress factor that must be overcome for successful freeze-drying of proteins.During the freezing process, as ice crystals are formed, the concentration of buffer salts and protein increase and the freeze concentration of these solutes can significantly affect the stability of proteins (Wang, 2005). Eto and Rubinsky (1993) showed that physiological concentrations of AFP significantly increase aqueous solution viscosity. The use of AFPs, along with cryoprotective compounds, might help protect enzymes and proteins during freeze-thawing.

Cryosurgery

Cryosurgery relies on the facilitation of intracellular ice formation by the presence of fish AFP. The mechanism of injury in cryosurgery has been categorized into immediate (physical) and delayed (biological) injury. Immediate injury is attributed to cell death that is caused by freeze-thawing of tissues, and delayed injury occurs as a result of the biological response to the freeze-thawing procedures. The addition of type I AFP is shown to enhance cryoinjury in primary human prostatic adenocarcinoma cells (Koushafar and Rubinsky, 1997). Muldrew *et al.* (2001) demonstrated that AFP can be used with clinical equipment and procedures to enhance primary cell injury in cryotherapy for prostate cancer. However, further studies may be essential for establishing these techniques effectively.

TRANSGENIC TECHNOLOGY

In Plants

The expression of AFPs in transgenic plants that possess the ability to cold acclimate will allow us to further test the hypothesis (Maunsbach *et al*., 2001) pertaining to (1) the ability of AFPs to cooperate with endogenous frost protection mechanisms and (2) the capacity of AFPs to protect nonacclimated plant tissues against freezing damage. The latter was shown by the expression of a carrot AFP gene in Arabidopis thaliana (Meyer *et al*., 1999) and N. tabacum(Worrall *et al*., 1998), resulting in the accumulation of antifreeze activity. Such studies are directed at assessing the efficiency of AFPs to confer frost resistance to commercially important plants (Ewart *et al*., 1999). Leaves of potato, canola, and A. thaliana plants were vacuum-infiltrated with AFP type I from winter flounder (Cutler *et al*., 1989). The use of transgenic technology to produce plants capable of synthesizing their own antifreeze has yielded transgenic tobacco, tomatoes, and potatoes (Hightower *et al*., 1991; Wallis *et al*., 1997; Worrall *et al*., 1998). Huang *et al*. (2002) expressed DendroidescanadensisAFP-1 in A. thaliana, which produced a transgenic plant with increased cold tolerance. On the basis of the magnitude of crop losses due to frost each year and the progressive adoption of exotic (and less hardy) species and the increasing acceptance of genetically modified organisms in the diet, the demand for increased cold tolerance is expected to grow in the coming years.

In Animals

Fletcher *et al*., 1986 showed that transgenic salmonids with the AFP gene would be able to produce endogenous AFPs and thus increase their freeze resistance. The highly active insect AFPs, which have higher activity (Graham *et al*., 1997), could be engineered in fishes to impart better cold tolerance. Development of freeze-resistant transgenic fishes will have a tremendous impact in aquaculture. Walker *et al.* (1995) expressed the fish AFP gene in *Drosophila* in order to develop a model for the generation of transgenic beneficial insects. Nicodemus *et al.* (2006) found that the activity of AFP in the hemolymph of transgenic *Drosophila* exhibited a supercooling point, which was slightly but significantly lower compared to wild type controls. Wang *et al.* (1995) showed that the assortment of gene of transgenic goldfish followed a Mendelian pattern and that the transgenic fishes were significantly more cold tolerant than the control.

Conclusion

The AFPs have potential industrial, medical, and agricultural application in different fields. There is great promise of application of AFP in foods that are frozen only for preservation. Cryopreservation of cells, tissues, and embryos is one of the most promising areas, with immense applications to both medical and biotech industries. Cryosurgery is emerging as a preferred method for treating cancers and tumors. Cold hardy transgenic plants and animals are also possible by using different molecular biological techniques with AFPs.

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