Antifreeze Proteins of Bacteria

M K Chattopadhyay

Antifreeze proteins (AFPs) prevent freezing of the body fluids of plants and animals inhabiting low temperature environments. Recently their role in cold adaptation of bacteria is also coming to the limelight. This article discusses some of these recent findings. Biotechnological importance of AFPs is also highlighted.

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Bacteria at Low Temperature

A couple of years ago, discovery of bacteria under a depth of about 3,600 meters ice sheet that covers Lake Vostok, a fresh water body in Antarctica, raised a lot of excitement among the scientific community. However, occurrence of bacteria in subzero temperatures of glaciers, polar regions and refrigerators is a well-known phenomenon. Investigations are in progress in several laboratories all over the world to elucidate the biochemical and genetic basis of the cold-tolerant nature of the organisms.

It has been found that the cold-hardy organisms possess some enzymes which are active at low temperatures (unlike similar enzymes obtained from temperate bacteria, which cease to act at low temperatures). These biocatalysts may hold the key to the mystery behind the ability of the producer organisms to survive and thrive in the extreme environment. They enable the producer cells to obtain food from the surroundings by the process of biodegradation, to continue the vital processes of life (viz. replication of DNA, synthesis of RNA from DNA and synthesis of proteins) and to maintain an optimum fluidity of the cell membrane that is required to perform its function at low temperature. The role of some carotenoid pigments in maintenance of optimum membrane fluidity in two Antarctic bacteria at low temperature has been postulated. Some special features of the RNA degradosome[1] has also been demonstrated. The body fluids of

[1] RNA degradosomes are protein complexes containing a set of exo- and endo ribonucleases and RNA helicase which coordinately degrade cellular RNAs and thereby regulate RNA abundance and gene expression.

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some wintering frogs and insects are known to contain sugars such as glucose and fructose, alcohols such as mannitol and glycerol and aminoacids such as alanine and proline. They are believed to stabilize cellular proteins and membranes at low temperature and hence called cryoprotectants. The cryoprotective role of glycine betaine (GB) in bacteria is beginning to be understood.

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Antifreeze Proteins: An Overview

Birds, fishes, amphibians, insects and plants have evolved several strategies to cope with extreme low temperature in their natural habitat. Mechanisms involved in their cold tolerance provide valuable clues to the studies on bacterial cold tolerance. One of the major strategies adopted by the cold-tolerant fishes, insects and plants is the production of specific proteins, which help them to maintain their body fluid in the liquid state at subzero temperatures. In general, they are called antifreeze proteins (AFPs), though other terms such as antifreeze glycoproteins, antifreeze polypeptides are also used to denote the structural features of these cryoprotectants.

Certain woody overwintering plants, which are unable to produce any AFP, can achieve a supercooling of their cellular fluid up to –50 ^{o}C. It is the structural features of the tissues such as tissue rigidity which enable them to do so. Some insects too can maintain their tissue fluid at –54 ^{o}C in the absence of any ice-nucleating agents and this is attributed to the presence of polyhydric alcohols. But supercooling is a very risky strategy since spontaneous and sudden freezing can cause extensive tissue injury. Production of AFPs on the other hand provides a safe strategy to avoid freezing at subzero temperatures.

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Mechanism of Action

We know that dissolved solutes in water causes a rise in its boiling point and depression in its freezing point. Thus sea water in cold regions freezes not at 0 ^{o}C but at –1.9 ^{o}C due to sodium chloride and other substances dissolved in it. In order to resist freezing of their blood, fishes inhabiting these harsh environments (e.g, Antarctic cod, Antarctic and Arctic eel pout, Arctic polar cod, Winter flounder) must have the freezing point of their blood below this temperature. However, low molecular weight dissolved solutes in the fish's blood cannot account for depression of freezing point by more than 1.4 ^{o}C. It is the AFPs which cause a further depression of freezing point of the blood by about 0.5 ^{o}C or more. Presence of some macromolecular antifreeze compounds in the blood of some Arctic fishes was detected by Scholander and his coworkers. Subsequent investigations by Arthur L.DeVries and his co-investigators revealed the protein nature of the antifreeze compounds in the blood of some Antarctic marine fishes.

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The depression caused by the low molecular weight solutes is colligative in nature. In other words, the degree of depression is a function of the number of solute molecules present in the system. The depression of freezing point caused by the AFPs is non-colligative in nature. Although freezing point is decreased, the melting point is not significantly altered. The difference between melting point and freezing point, called Thermal Hysteresis (TH), is a measure of the antifreeze activity. This mechanism is also known as freeze avoidance. According to the adsorption inhibition mechanism proposed by Raymond and DeVries, irreversible adsorption of AFPs on the ice crystal surface makes ice grow as convex fronts between the protein molecules. The pattern is thermodynamically unfavorable, which in turn leads to the antifreeze effect.

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It is evident by this time that AFPs isolated from different sources vary widely in their potency. The value of TH (a difference of 1.4 ^{o}C between melting point and freezing point) obtained by using 20 µM of an insect AFP was found to be associated with more than a 100-fold higher concentration of a fish AFP.

Besides causing TH, the AFPs act by another mechanism called freeze tolerance. In most of the freezing conditions, formation of ice takes place as a multicrystalline mass. Growth of large ice

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crystals occurs at the expense of smaller crystals, a phenomenon termed ice recrystallization. AFPs limit the growth of ice crystals at sub-zero temperatures by being adsorbed on the ice surface. This mechanism is known as recrystallization inhibition (RI).

In addition to lowering of the freezing point of the body fluid and preventing damage caused by ice recrystallization, AFPs in some cases are believed to protect membranes from cold-induced damages by inhibiting thermotropic phase transitions and preventing leakage by blocking ion channels. Thus, their cryoprotective potential is contributed by a number of factors.

Evolutionary Aspects

Evolutionary studies on AFPs present a baffling scenario. One AFP that occurs in an Antarctic fish, appears to have evolved from a pancreatic trypsinogen-like protease. Some plant AFPs are known to be homologs of some plant pathogenesis proteins. The AFPs are found to retain enzyme activity but the homologs have no antifreeze activity. These AFPs may represent an intermediate stage in evolution with the original function being retained. It appears that a series of very different proteins have independently evolved a common function (i.e., ice binding) to form AFPs, despite having no sequence similarity. There is no consensus ice-binding motif of AFPs, making PCR-based studies on them impossible.

Bacterial AFPs

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The presence of TH activity in bacteria was demonstrated for the first time by Duman and Olsen. Since then, a number of cold-adapted bacteria (*Pseudomonas putida*, *Pseudomonas fluorescens*, *Marinomonas protea*, a *Moraxella* species) have been found to possess AFPs. A sizeable fraction of them consists of Antarctic isolates. Thus the role of AFPs in cold adaptation of bacteria is indicated.

In general, high values of TH are found to be associated with AFPs isolated from insects (3-6 ^{o}C) and fishes (0.7 to 1.5 ^{o}C) whereas AFPs isolated from plants and bacteria are known to have lower values of TH (0.2 to 0.5 °C in case of plant AFPs and less than 0.1 °C in case of bacterial AFPs). Hence it is believed that AFPs in animals work by the mechanism of freeze-avoidance whereas freeze-tolerance is the strategy used by the AFPs occurring in plants and bacteria.

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Recent Findings

Two recent reports reveal a breach in this widely believed notion. In one investigation, concentrated supernatant of the cell lysate of a bacterium *Marinomonas primoryensis*, isolated from a saline and permanently cold Antarctic lake, was found to cause over 2 °C freezing point depression, which is higher than the TH value of most of the AFPs isolated from fishes. The activity was reduced in the presence of EDTA but could be restored by saturation of EDTA with calcium chloride. Thus, the antifreeze activity was Ca^{2+} dependent, a feature known so far to be associated with fish AFPs but not with bacterial AFPs.

Subsequently, a cell-free extract of an Antarctic strain *Flavobacterium xanthum* IAM 12026, has been found to have both freezing point depression and ice recrystallization-inhibiting activities. The purified protein was found to have a TH value of 1.19 °C.

These reports indicate that like fish and insect AFPs, bacterial AFPs can also act by the mechanism of freeze avoidance.

Biotechnological Aspects

AFPs appear to be useful in cryosurgery and also in the cryopreservation of whole organisms, isolated organs, cell lines and tissues. In food industry, AFPs can be used to improve the quality of frozen food. Improved cold tolerance in fishes has been achieved in some cases by direct injection of AFPs and in another case by transgenic expression of an AFP. In refrigeration industry, AFPs appear to be useful in maintaining the fluidity of ice slurry that is used as secondary refrigerant. However, the commercial potential of AFPs remains underutilized by and large till now.

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Future Studies

The recent reports on bacterial AFPs with high TH activity, clearly indicate that bacterial AFPs offer a challenging area for investigation. They are also potential candidates for industrial use. Isolation of AFPs from many other cold-adapted bacteria and studies on their mode of action may provide insight into the intricacies of cellular machinery involved in stress adaptation.

Suggested Reading

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