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> ONE MONTH, ONE PROTEIN <

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ice whisperer

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No one likes the cold. Humans wear scarves, fur boots, quilted coats and woollen hats to keep the harshness of winter out while other creatures grow their own fur or line their bodies with a thick layer of blubber. There are those, too, who have a more subtle approach to dealing with extreme temperatures and, instead of sporting a protective coating of fur or fat, tame the cold by acting upon its actual source. As an example, various species of fish or bacteria who live in Arctic environments have evolved systems to keep ice crystals from developing further inside them or sometimes even in the surrounding extracellular medium. For what reason? Because ice crystals can damage cell membranes by puncturing them or causing them to rip, which is life threatening. One particular Arctic yeast known as *Leucosporidium* has developed a system that – in freezing conditions – hinders the growth of ice crystals by secreting a protein that binds to them and ultimately lowers their freezing point. This particular protein has been coined *Leucosporidium* ice-binding protein, or more simply LeIBP.

Silkscreen print 2015, by Anaëlle Clot

Courtesy of the artist

The first antifreeze proteins (AFPs) were isolated from Antarctic teleost fish in the 1970s by the American animal biologist Arthur DeVries. Since then, many other AFPs have surfaced in organisms as varied as insects, plants, bacteria, fungi, diatoms and algae and, nowadays, scientists prefer to talk about the more generic ice-binding proteins, or IBPs, to which antifreeze proteins belong. Besides anti-freeze properties, other IBPs include ice-nucleation proteins that actually promote the growth of ice crystals, or ice adhesion proteins whose role is to anchor something to ice crystals or even organise their structure. The role of IBPs is not limited to surviving extreme temperatures either: many organisms produce IBPs to gain access to nutrients for instance. As an illustration, one species of Antarctic bacterium uses IBPs to bind to ice in the upper regions of sea lakes where there is oxygen and sustenance, and various plant pathogens use IBPs to initiate the formation of ice crystals in the epithelia of fruit, thus literally digging a passageway to food.

To which category does the Arctic yeast's IBP belong? LeIBP is an ice-clinger; secreted into the extracellular medium by *Leucosporidium*, its role is to lower the environment's freezing point. How? An initial adsorption-inhibition model was introduced in the 1970s, which hypothesised that AFPs bind irreversibly to ice crystals thus forcing them to continue their growth between the adsorption sites – much in the way a cake mixture rises within the confined limits of a baking tin. As a result, the ice ends up curving, which has the concomitant effect of lowering the freezing point. This shift in the freezing point creates a temperature interval – known as the thermal hysteresis gap – which keeps the yeast's immediate vicinity in a delicately-balanced viable state where ice crystals neither grow nor melt. The model has since been refined and takes into account other parameters, such as the nature of growth on rough prism planes and the polymeric nature of IBPs.

The structure of LeIBP is similar to that of all known hyperactive antifreeze proteins, i.e. an association of three parallel beta strands twisted into a helical pattern – a structure also known as a beta helix. The active form of LeIBP seems to be as a homodimer where each subunit is barrel-like in form and provides an ice-binding platform that has a flat binding surface. Unlike other IBPs, a C-terminal tail protrudes from each LeIBP and seems to strengthen the interaction of the two subunits forming the homodimer. LeIBP is also glycosylated, and although glycosylation alone does not seem to be necessary for LeIBP activity, scientists believe that dimerization and LeIBP glycosylation are both physiologically relevant. It may be that glycosylation contributes to LeIBP folding and secretion.

How do LeIBPs actually dock to ice crystals? Though in many ways similar to other antifreeze proteins, LeIBP's ice-binding motif is more complex and diverse. This would explain why the Arctic yeast IBP is able to bind to a variety of basal and prism planes of ice crystal lattices and promotes a broad spectrum of interactions. Additional side chains can also reorient LeIBPs with respect to crystals, thus nurturing yet additional ways of clinging to ice. When LeIBP actually docks to nascent ice crystals, it undergoes no major conformational change, which implies that binding is probably achieved by way of a combination of parameters including hydrogen bonding, structural matching with the ice lattice and the inclusion of methyl entities and their effect within the ice lattice.

How IBPs preserve organisms from the drawbacks of freezing temperatures has quite naturally inspired the food industry. IBPs could enhance food shelf-life by postponing cell damage for instance. Transgenic fish and crops resistant to frost have been engineered, but there are concerns about their impact on the environment and human health. IBPs could also prove to be very useful in the cryopreservation of cells, tissues, embryos and organs but their underlying mechanisms are still too poorly understood, and the various tissues could still undergo damage or perhaps even necrosis. Other applications involve the creation of special materials whose morphology could be tailored using ice templates – such as porous carrier material for drug delivery or scaffolds for tissue engineering. The applications seem to be numerous. To date, however, the commercial application of IBPs is limited to ice cream to keep the texture smooth and – to end on a more poetic and seasonal note – to…the making of snow.

Cross-references to UniProt

Ice-binding protein, *Leucosporidium* (Arctic Yeast) : C7F6X3

References

1. Lee J.H., Park A.K, Do H., Park K.S., Moh S.H., Chi Y.M.
   Structural basis for antifreeze activity of ice-binding protein from Arctic yeast
   Journal of Biological Chemistry 287:11460-11468(2012)
   PMID: 22303017

2. Voets I.K.
   From ice-binding proteins to bio-inspired antifreeze materials
   Soft Matter 13:4808-4823(2017)
   PMID: 28657626