

## Cool news

Vivienne Baillie Gerritsen

**T**he antifreeze you load your window screen with on a cold frosty morning is nothing new. Antarctic marine fish devised a way to prevent themselves from freezing to death long before the Model T Ford had been thought up. While some organisms use sodium chloride, potassium, calcium, urea, free amino acids or glycerol to survive harsh conditions, the Antarctic notothenioids – such as the yellowbelly rockcod – have developed sophisticated ways of coping with polar conditions by producing a form of high-class antifreeze: antifreeze glycopeptides. These antifreeze proteins are more than 100 to 200 times more effective than any other type of antifreeze molecule and are found in most notothenoid body fluids, which is fortunate since these fish spend their lives in ice-laden waters. Fish that live in more northern temperate waters produce antifreeze proteins on a seasonal basis.

A fish cannot avoid swallowing ice particles in seawater which is burdened with them. The trick then is to prevent the ice crystals from growing any further. How does an antifreeze protein work? The prevailing theory is one of polarity. Antifreeze glycoproteins found in the Antarctic notothenioids have a primary structure based on the repetition of glycotripeptides (Thr-Ala/Pro-Ala)<sub>n</sub> with a sugar attached to each threonine. Each triplet is slightly polar and it has been suggested that they cling to the polar water molecules thus coating the budding crystal.



The yellowbelly rockcod

Courtesy of 3Way Ocean Charters

Such “clinging” would be in the form of hydrogen-bonding between the hydroxyl, carboxyl and amino groups of the antifreeze proteins and the oxygen, or hydrogen, in the ice

lattice. The antifreeze proteins then fold and position themselves in such a way that one side of the chain binds to water molecules and the other does not. And, as a consequence, the growth of the ice crystal seed is interrupted.

Large molecules with repeating units are more effective than small ones because they can form more hydrogen bonds with the ice lattice. Providing, of course, that the spatial arrangement of the tripeptides within the antifreeze molecule follows that of the water molecules in the ice lattice... It may well be that the peptide is completely extended, in which case the disaccharides would provide a form of backbone and the carbonyl groups on the peptide would be separated by 7.3Å – which just happens to be the distance that separates alternate oxygen atoms along the c-axis of the ice lattice.

Antifreeze proteins could also clog up holes that are formed when the fish lose heat. Indeed, in the process of cooling, molecules clump together leaving open patches. This is not desirable since water can pour through the holes. Antifreeze proteins could play a role in coating the cells, thus making them waterproof and saving the fish from certain death.

The genetics of the Antarctic notothenioids are particularly interesting. The tripeptides are believed to have evolved from a small integral part of an ancestral digestive protein, which could bind ice. This is hardly surprising since it

would have been more than useful to have some type of antifreeze in the digestive tract. Evolution would have taken care of the rest and permitted the *Notothenioidei* suborder to survive the first freezing of the southern oceans. What is more, these seas grew colder about 10 to 14 million years ago and the sequence divergence occurred 5 to 14 million years ago.

The use of antifreeze is not uncommon. It is a way of preserving an organism in a cooled state without destroying tissues for instance. Currently, only very few human tissues are frozen. Sperm is one, although more than one third is lost in the process. Fish antifreeze has been introduced into crop plants to protect them

from frost. However, it has been discovered since that the carrot antifreeze protein is far more effective and stable.

And there is the world of cryopreservation. Besides the preservation of a human being in its entirety, antifreeze proteins could be used to preserve organs – which do not survive more than a few hours – prior to transplant operations so that one can simply go and pick them in the fridge when needed. The same could be imagined for chicken legs in the supermarket. Prospects of cryosurgery are also promising: chemotherapy could be carried out on kidney tumours, for instance, while the patient survives on a dialysis machine.

## Cross-references to Swiss-Prot

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