Bacterial Cryoprotectants

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Cold-adapted organisms are known to accumulate cryoprotective substances inside their cells. Glycine betaine is a bacterial cryoprotectant. It is believed to act by stabilizing cellular proteins and membranes at low temperature.

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Life sustained at near-zero or sub-zero temperatures, poses a riddle to biologists. Diverse forms of living entities ranging from bacteria to insects, fishes, frogs and birds are known to survive in the extremely cold climate of polar regions and high mountain forests. A peep into the mechanisms of how they have adapted to withstand extreme cold can provide us valuable clues to some of the mysteries of life. The knowledge obtained from such investigations can also be exploited for cryopreservation of organs or tissues for medical purposes, and may also offer some tips to scientists striving to find a way to control food-borne pathogens that survive at low temperatures. Moreover, the physiology of cold-adapted organisms may help us evaluate the possibility of life on distant frozen planets.

What are Cryoprotectants?

Substances which play a role in the cold-tolerance exhibited by the organism producing them, are called cryoprotectants. It has been known that organisms use several strategies to survive at low temperature. For example, in one experiment the wood frog, which hibernates in the icy areas of North America, was found to have an extremely high level of blood glucose of ~ 550 millimoles per litre during winter. By contrast, normal blood glucose level in these amphibians ranges from 1 to 5 millimoles per litre, and a fasting human with a value above 6.5 millimoles per litre is considered a diabetic person. Some other frogs accumulate glycerol instead of glucose in their blood. Glycerol is also the cryoprotectant found in the hemolymph of some insects. Other

Keywords
Thermal adaptation, glycine betaine, bacteria, heat shock proteins, chemical chaperones.

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Glycine Betaine – A Bacterial Cryoprotectant

The role of glycine betaine (henceforth, betaine) as a bacterial cryoprotectant was first demonstrated in the food borne pathogen *Listeria monocytogenes*. This organism survives at low temperature and high osmolarity (paradoxically these are the two conditions that we use for preservation of food materials). It was shown by a group of investigators at the University of California, Davis, USA, that, following incubation at 7°C for 32 days in presence of betaine, more than 100 colonies appeared in the culture plate, whereas no growth was detected under similar conditions without betaine. They also observed that uptake of betaine into the bacterial cell was enhanced at low temperature. Efficient accumulation of betaine by *L. monocytogenes* at low temperature was subsequently found to be mediated by a protein called sigma B, which is a part of RNA polymerase, the enzyme which plays the key role in bacterial gene expression. Sigma B in *L. monocytogenes* is known to get more activated in a high osmolar and low temperature environment. Very recently, the role of an ATP-dependent transport system in the uptake of betaine during cold stress has also been demonstrated.

The cryoprotective effect of betaine in a cyanobacterial strain was shown a couple of years ago by genetic manipulation. The enzyme choline oxidase, encoded by the gene *cod A*, catalyzes oxidative transformation of choline into betaine. A team of investigators led by N Murata in the National Institute for Basic Biology, Okazaki, Japan, successfully transformed a cyanobacterial strain (*Synechococcus* PCC 7942) with *cod A* from another bacterium, *Arthrobacter globiformis*. The transformed cells were found to accumulate high intracellular levels of betaine and were able to grow at 20°C, a temperature at which the growth of the non-transformed cells was suppressed. Following exposure to strong light, photosynthesis is inhibited in oxygen-producing photosynthetic organisms (like cyanobacteria), and this inhibition becomes much more pronounced at low temperature. In the presence of a high intracellular concentration of betaine, the ability of the cyanobacterial strain to recover from low temperature-induced photoinhibition was improved.

Mechanism of Action

Betaine is a well-known bacterial osmoprotectant i.e. a compound which stimulates the growth of bacteria in high osmolar condition. Many of these compounds are not catabolized or incorporated into macromolecules. Though most of the studies on bacterial cryoprotectants have been performed on betaine, its mechanism of action is not yet clearly known. It must be mentioned in this context that it is not only a cryoprotectant and osmoprotectant, but has also been shown to have a thermoprotective effect in *Escherichia coli*. Does this imply that betaine is a general stress-protectant in bacteria? Evidence available in the literature suggests otherwise. In a recent study, when a strain of *Enterococcus faecalis* was treated with sodium chloride, it developed some tolerance to bile salt. However, this bile salt tolerance was substantially reduced in the presence of betaine, although it could reverse growth inhibition by sodium chloride. So betaine is certainly not a saviour in all sorts of stress conditions.

Betaine is believed to act as a cryoprotectant by virtue of its ability to behave as a chemical chaperone. Chemical chaperones are low molecular weight compounds that suppress the aggregation of cellular proteins during stress conditions (for details see Research News, *Resonance*, Vol. 6, No.5, 2001). An interesting study performed by K-C Chow and W L Tung of Hong Kong University in 1998 showed that when an *E. coli* strain was stored at –80°C for 24 hours only 0.4% of the original population could survive. However when the cells were exposed to 42°C for 30 minutes prior to freezing, viability was raised to almost 4%.

Suggested Reading

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[4] V Pichereau, S Bourot, S Flahaut, C Blanco, Y Auffray and T Bernard, The osmoprotectant glycine betaine inhibits salt-induced cross-tolerance towards lethal treatment in *Enterococcus faecalis*, *Microbiology*, Vol.145, pp. 427-435, 1999.

[5] K-C Chow and W L Tung, Overexpression of dna K/ dna J and gro EL confers freeze tolerance to *Escherichia coli*, *Biochemical and Biophysical Research Communications*, Vol. 253, pp. 502-505, 1998.

They also showed that some heat shock proteins (*Box* 1) were synthesized in the heat-treated cells. Those proteins are believed to protect bacterial cell from thermal stress. How can we relate their synthesis with improved cold-tolerance? The investigators postulated that the clue lies in the ability of the heat shock proteins to maintain the correct folding of the cellular proteins both during heat and cold stress. Following the same line of logic, it seems possible that chaperoning effect of betaine enables it function as an osmoprotectant, thermoprotectant and cryoprotectant.

The possibility of the involvement of betaine in regulation of membrane fluidity also cannot be ruled out. With lowering of environmental temperature, the cell membrane tends to become rigid, and bacterial cells are known to adopt several measures to maintain an optimum membrane fluidity. Biochemical changes leading to the incorporation of unsaturated, short-chain and branched-chain fatty acids into the lipids, in response to a decrease in temperature, are well-documented. The process, as a whole, is called homeoviscous adaptation of membrane fluidity. It was shown a couple of years ago that when two strains of *Listeria monocytogenes* were grown at low temperature in presence of betaine, the amount of a branched-chain fatty acid in the cell was increased. It was not only the predominant component in the fatty acid profile of the strains, it was also crucially essential for their survival in cold. A mutant, unable to grow at low temperature and deficient in this branched-chain

Box1. Heat Shock Proteins (HSPs)

Activation of some genes in response to a temperature upshock was first noticed in the fruit fly *Drosophila melanogaster* in 1962. The products of the genes were identified and the term HSPs was coined in 1974. However, it is now known that the term HSPs is a misnomer since these proteins, present in all species studied, can be induced not only by heat shock but also by other stresses like oxidative stress, nutritional deficiencies, ultraviolet irradiation, exposure to some chemicals (e.g ethanol) and viral infection. Under normal conditions, some of the HSPs function as molecular chaperones and proteases. Thus, they help correct the folding of the cellular proteins and degradation of damaged proteins.They appear to confer thermotolerance to the producer cell.

fatty acid, was found to have a less fluid membrane than the parent strain. When the mutant was grown in presence of a precursor of branched-chain fatty acids, the membrane fluidity and cold-tolerance associated with the parent strain were restored. Thus, it appears that betaine may allow cold tolerance in bacteria by promoting the synthesis of membrane fluidizing fatty acids. Further investigations with some other cold-adapted bacteria may help to confirm this hypothesis. Studies on plants and animals indicate that, besides stabilizing cellular proteins and membrane at low temperatures, cryoprotectants may also act by:

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(1) lowering the freezing point of intracellular water, (2) reducing the loss of intracellular water, (3) increasing the intracellular content of bound water, and (4) modifying the growth of ice crystals in the extracellular environment.

Conclusion

At the present time, betaine serves as a model for the study of bacterial cryoprotectants. However, we need to extend our knowledge in this area by studying some other potential candidates (like glycerol). Attention should be focussed on molecules which are available in the metabolic pool and have cryoprotective properties (like proline), or substances which are precursors of cryoprotectants (like choline). Studies involving cold-adapted bacteria obtained from various natural sources, are also likely to generate new clues eventually leading to a better understanding of the phenomenon of cryoprotection.

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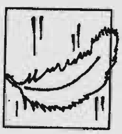

"There is for me powerful evidence that there is something going on behind it all .... It seems as though somebody has fine-tuned nature's numbers to make the Universe .... The impression of design is overwhelming."

Paul Davies
(British astrophysicist)