Design and engineering of ice-binding proteins using phage display

Koli Basu[1], Robert L. Campbell[1], Ido Braslavsky[2] and Peter L. Davies[1]

[1]*Department of Biochemistry, Queen's University, Kingston, Ontario, Canada*

[2]*Institute of Biochemistry, Food Science and Nutrition, Faculty of Agricultural, Food and Environment, the Hebrew University of Jerusalem, Rehovot 76100, Israel*

Given the significant diversity in antifreeze protein (AFP) sequences and structures, it is challenging to determine what the essential elements are for ice-binding activity. By engineering non-AFPs to demonstrate AFP activity, the requirements for protein-to-ice adsorption can be better understood. Phage display is frequently used to determine the epitopes involved in protein-ligand interaction because the binding molecule is directly linked to the genetic information that specifies it. The major coat protein of the M13 bacteriophage, P8, is a 5.2-kDa alpha-helix. The essential residues required for its function as a coat protein have previously been mapped by alanine-scanning mutagenesis. The outer residues towards the N terminus appear to be the least constrained and we are altering these to include elements of the ice-binding site of type I AFP. The structural similarities between P8 and type I AFP should allow for a merger of these proteins. The resulting bacteriophage will include the engineered P8 in its protein coat and will be tested for its ability to bind to ice. There are 2700 copies of the P8 protein in the bacteriophage coat and we anticipate that even weak ice-binding activity might be compensated for by the large numbers of proteins available for binding.

Supported by the Canadian Institutes for Health Research