Structural adaptation of enzymes to low temperatures

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It is estimated that around 90% of the biosphere exists at temperatures below 10*C. Indeed, the earth's surface is dominated by low temperature environments such as Artic and Antarctic continents, mountains regions, and the marine waters which cover 70% of its area and display, below 1000 m under the sea level, temperatures not exceeding 5*C. Psychrophilic organisms live at such low temperatures, where most of the other species cannot grow and to survive they need to produce enzymes able to perform efficiently their catalysis in these extreme environmental conditions. At the same temperatures, enzymes from mesophilic or thermophilic organisms are generally unable to sustain a viable metabolism. For these reasons, enzymes synthesized by psychrophilic organisms have considerable biotechnological potential. Whereas important progress has been made in elucidating the molecular adaptation mechanism of enzymes produced by extremophiles such as hyperthermophiles, the molecular basis of cold adaptation are still relatively poorly understood. Recent accumulation of structural data on psychrophilic enzymes is beginning to shed light on their functional and structural characteristics The commonly observed features of these cold active enzymes are their increased catalytic efficiency at low temperatures measured as kcat/KM and a significantly increased thermolability which is believed to be consequence of enhanced peptide chain flexibility. The amount of available structural data, primary and tertiary, on psychrophilic enzymes is now sufficient to undertake a comprehensive and significant comparative analysis. Studies on the structural adaptation of thermophilic enzymes utilized comparative analysis.

We carried out a comparative analysis of 21 psychrophilic enzymes belonging to different structural families from prokaryotic and eukaryotic organisms. The sequences of these enzymes where multiply aligned to 427 homologous proteins from mesophiles and thermophiles. The net flux of amino acid exchanges from meso/thermophilic to psychrophilic enzymes was measured. To assign the observed preferred exchanges to different structural environments, like secondary structure, solvent accessibility and subunit interfaces, homology modeling was utilized to predict the secondary structure and accessibility of aminoacid residues for the psychrophilic enzymes for which no experimental three-dimensional structure is available. Our results show a clear tendency for the charged residues Arg and Glu to be replaced at exposed sites on a-helices by Lys and Ala, respectively, in the direction from "hot" to "cold" enzymes. Val is replaced by Ala at buried regions in a-helices. Compositional analysis of psychrophilic enzymes shows significant increase of Ala and Asn and decrease of Arg at exposed sites. Buried sites in b-strands tend to be depleted of Val. The preferred amino acid exchanges observed from thermo/mesophiles to psychrophiles and compositional analysis indicate a decreased number of sidechain potential H-bonds and salt bridges in cold-adapted enzymes. To this respect, strategy of cold adaptation seems to use the same principle as the hot adaptation, namely increase of the number of H-bonds/ion pairs going from low to high temperatures. The results suggest possible general rules for protein engineering experiments aimed at producing enzymes catalytically effective at low temperatures. It should be emphasized that the statistical analysis described in this work can only detect general features of enzyme cold adaptation while it overlooks the subtle structural modifications that can be reliably identified by detailed intra-family structural comparison.