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Analysis of surface charged residues involved in thermal stability in *Alicyclobacillus acidocaldarius* esterase EST2

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Esterases, lipases and cholinesterases belong to a superfamily of phylogenetically related proteins with representatives in Eukarya, Bacteria and Archaea. Among these we have studied some thermostable members of the Hormone Sensitive Lipase family. The thermophilic esterase EST2 from *Alycyclobacillus acidocaldarius* shows high catalytic activity (about 7000 U/mg on p-nitrophenyl-esters), and promiscuous activities on thioesters, acyl-glycerol esters, vinyl esters and acetylated sugars. From a biotechnological point of view the enzyme is interesting as active component of a biosensor against organophosphate pesticides and in the hydrolysis or synthesis of industrially interesting esters. Here it is reported a comprehensive analysis through alanine-scanning mutagenesis of the contribution of surface ion pairs to the thermal stability of EST2, which are involved also in substrate specificity. Several mutants have been produced as single and double mutants corresponding to selected ion pairs; furthermore, residues of a large ion network on the protein surface have been changed to disrupt the network. The study of the individual factors involved in thermostability and their structural interpretation reveals that the high stability of this thermophilic protein can be explained by the contribution of a few residues at the protein surface. Comparative analyses of EST2 with the homologous hyperthermophilic esterase AFEST from *Archaeoglubus fulgidus* have suggested some surface residues that could potentially increase the thermal stability of EST2. Accordingly, the site-direct mutagenesis of these residues led to obtain a variant of EST2 with increased thermostability compared to the wild type enzyme. A further characterization of these mutants will be reported.

Biography

Luigi Mandrich is currently a staff researcher at the Institute of Protein Biochemistry of the National Research Council. He graduated in Biological Sciences at the University of Naples "Federico II", and in 2004 received the PhD in Industrial Biotechnology.During his training he moved to The Netherland and Argentina to exploit new approaches to the use of exogenous enzymes in cheese making. He has carried out research in the field of biochemistry, and enzymes biotechnological applications. He is involved in research projects concerning the technology of recombinant DNA to produce and study human and bacterial proteins involved in detoxification of pesticides and to counteract pathogens infections. He is co-author of more than 40 papers on international peer reviewed journals, and he is Editor of the "Cloning and Transgenesis" journal.

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