

Role of key salt bridge residues in *Geobacillus thermodenitrificans* carboxylesterase EstGtA2: Fine-tuning activity at low temperature

Marc Beauregard and David M. Charbonneau
Lignocellulosic Materials Research Center, Canada

The *G. thermodenitrificans* carboxylesterase EstGtA2 (access no. AEN92268) forms, with other bacterial homologs, a novel family of thermostable lipolytic enzymes named N'. Members of the family hydrolyze a wide variety of biotechnologically relevant ester molecules. EstGtA2 is optimally active at 50-65°C and shows a highly pH-dependent thermostability profile with T_m ranging from 55-69°C between pH 4 and 10. Five selected salt bridge-forming arginine and lysine residues exclusively conserved in the N' family were analyzed using a combinatorial alanine-scanning approach to determine their role in EstGtA2 thermostability. A set of 14 (R/K→A) mutants was produced, five single, three double, three triple and three quadruple mutants corresponding to the following ion-pairs (E3/R54, E12/R37, E66/R140, D124/K178 and D205/R220). We found that all of these mutants but one was folded and active at 25°C, indicating very high tolerance to non-conservative mutations (R/K→A). All mutations were destabilizing, decreased apparent T_m by 8 to 14°C. Among the three quadruple mutants studied, one did not adopt the typical α/β hydrolase fold and is completely inactivated at 25°C. Single mutations led to an increase in activity at low temperature compared to wild type and may be used for fine tuning of EstGtA2 activity at low temperature. We also identified a particular salt bridge which links two loops of the active site, located $i - 2$ and $i - 4$ residues from the catalytic Asp and His respectively. This bridge is conserved in other distantly related bacterial hydrolases but its polarity is another hallmark of N' family. We demonstrated the role of H222 in controlling activity-pH dependence, by introducing a His to Arg mutation. The consequent pK_a shift did not affect activity-pH response, but was found to improve EstGtA2 thermostability under alkaline pH. Residue 222 can be used as pH switch for optimization of EstGtA2 for specific biotechnological purposes, but its importance for the nearby active site appears limited.

Biography

Marc Beauregard has completed his Ph.D. (Biophysics) in 1989 at Université du Québec à Trois-Rivières (Canada). He then held postdoctoral fellowships at Max-Planck-Institute, Université de Liège and with Agriculture Canada. Since the beginning of his career, he has been very active in technology transfer and R&D, contributed to development of commercialized biotechnology products, and has been consulting with several technology-driven and life science companies in Quebec. He is a professor with UQTR since 1998, has published 55 papers and is a member of PROTEO and CRIBIQ (protein and biotechnology networks).

Marc.Beauregard@uqtr.ca