Relevance of local flexibility near the active site for enzymatic catalysis : biochemical characterization and engineering of cellulase Cel5A from bacillus agaradherans

Saavedra, J. M., Azócar, M. A., Rodríguez, V., Ramírez-Sarmiento, C. A., Andrews, B. A., Asenjo, J. A., & Parra, L. P. (2018). Relevance of Local Flexibility Near the Active Site for Enzymatic Catalysis: Biochemical Characterization and Engineering of Cellulase Cel5A From Bacillus agaradherans. *Biotechnology journal*, *13*(8), 1700669. <10.1002/biot.201700669> Accessed 17 Nov 2020.

Abstract

Detailed molecular mechanisms underpinning enzymatic reactions are still a central problem in biochemistry. The need for active site flexibility to sustain catalytic activity constitutes a notion of wide acceptance, although its direct influence remains to be fully understood. With the aim of studying the relationship between structural dynamics and enzyme catalysis, the cellulase Cel5A from *Bacillus agaradherans* is used as a model for in silico comparative analysis with mesophilic and psychrophilic counterparts. Structural features that determine flexibility are related to kinetic and thermodynamic parameters of catalysis. As a result, three specific positions in the vicinity of the active site of Cel5A are selected for protein engineering via site-directed mutagenesis. Three Cel5A variants are generated, N141L, A137Y and I102A/A137Y, showing a concomitant increase in the catalytic activity at low temperatures and a decrease in activation energy and activation enthalpy, similar to cold-active enzymes. These results are interpreted in structural terms by molecular dynamics simulations, showing that disrupting a hydrogen bond network in the vicinity of the active site increases local flexibility. These results provide a structural framework for explaining the changes in thermodynamic parameters observed between homologous enzymes with varying temperature adaptations.

Keywords

Cellulase; Flexibility; Molecular dynamics; Protein engineering