Enhancing the stability of Geobacillus zalihae T1 lipase in organic solvents and insights into the structural stability of its variants

ABSTRACT

Critical to the applications of proteins in non-aqueous enzymatic processes is their structural dynamics in relation to solvent polarity. A pool of mutants derived from Geobacillus zalihae T1 lipase was screened in organic solvents (methanol, ethanol, propanol, butanol and pentanol) resulting in the selection of six mutants at initial screening (A83D/K251E, R21C, G35D/S195 N, K84R/R103C/M121I/T272 M and R106H/G327S). Site-directed mutagenesis further yielded quadruple mutants A83D/M121I/K251E/G327S and A83D/M121I/S195 N/T272 M, both of which had improved activity after incubation in methanol. The km and kcat values of these mutants vary marginally with the wild-type enzyme in the methanol/substrate mixture. Thermally induced unfolding of mutants was accompanied with some loss of secondary structure content. The root mean square deviations (RMSD) and B-factors revealed that changes in the structural organization are intertwined with an interplay of the protein backbone with organic solvents. Spatially exposed charged residues showed correlations between the solvation dynamics of the methanol solvent and the hydrophobicity of the residues. The short distances of the radial distribution function provided the required distances for hydrogen bond formation and hydrophobic interactions. These dynamic changes demonstrate newly formed structural interactions could be targeted and incorporated experimentally on the basis of solvent mobility and mutant residues.

Keyword: Lipases; Organic solvent; Mutagenesis; Stability; Molecular simulations