Facile modulation of enantioselectivity of thermophilic Geobacillus zalihae lipase by regulating hydrophobicity of its Q114 oxyanion

ABSTRACT

Site-directed mutagenesis of the oxyanion-containing amino acid Q114 in the recombinant thermophilic T1 lipase previously isolated from *Geobacillus zalihae* was performed to elucidate its role in the enzyme□s enantioselectivity and reactivity. Substitution of Q114 with a hydrophobic methionine to yield mutant Q114M increased enantioselectivity (3.2-fold) and marginally improved reactivity (1.4-fold) of the lipase in catalysing esterification of ibuprofen with oleyl alcohol. The improved catalytic efficiency of Q114L was concomitant with reduced flexibility in the active site while the decreased enantioselectivity of Q114L could be directly attributed to diminished electrostatic repulsion of the substrate carboxylate ion that rendered partial loss in steric hindrance and thus enantioselectivity. The highest E-values for both Q114L (E-value 14.6) and Q114M (E-value 48.5) mutant lipases were attained at 50 °C, after 12□16 h, with a molar ratio of oleyl alcohol to ibuprofen of 1.5:1 and at 2.0% (w/v) enzyme load without addition of molecular sieves. Pertinently, site-directed mutagenesis on the Q114 oxyanion of T1 resulted in improved enantioselectivity and such approach may be applicable to other lipases of the same family. We demonstrated that electrostatic repulsion phenomena could affect flexibility/rigidity of the enzyme-substrate complex, aspects vital for enzyme activity and enantioselectivity of T1.

Keyword: T1 lipase; Geobacillus zalihae; 2DSN; Site-directed mutagenesis; Enantioselectivity; Oxyanion