Effects of lid 1 mutagenesis on lid displacement, catalytic performances and thermostability of cold-active Pseudomonas AMS8 lipase in toluene

ABSTRACT

Pseudomonas fluorescens AMS8 lipase lid 1 structure is rigid and holds unclear roles due to the absence of solvent-interactions. Lid 1 region was stabilized by 17 hydrogen bond linkages and displayed lower mean hydrophobicity (0.596) compared to MIS38 lipase. Mutating lid 1 residues, Thr-52 and Gly-55 to aromatic hydrophobic-polar tyrosine would churned more side-chain interactions between lid 1 and water or toluene. This study revealed that T52Y leads G55Y and its recombinant towards achieving higher solvent-accessible surface area and longer half-life at 25 to 37 °C in 0.5% (v/v) toluene. T52Y also exhibited better substrate affinity with long-chain carbon substrate in aqueous media. The affinity for pNP palmitate, laurate and caprylate increased in 0.5% (v/v) toluene in recombinant AMS8, but the affinity in similar substrates was substantially declined in lid 1 mutated lipases. Regarding enzyme efficiency, the recombinant AMS8 lipase displayed highest value of kcat/Km in 0.5% (v/v) toluene, mainly with *p*NPC. In both hydrolysis reactions with 0% and 0.5% (v/v) toluene, the enzyme efficiency of G55Y was found higher than T52Y for *p*NPL and *p*NPP. At 0.5% (v/v) toluene, both mutants showed reductions in activation energy and enthalpy values as temperature increased from 25 to 35 °C, displaying better catalytic functions. Only T52Y exhibited increase in entropy values at 0.5% (v/v) toluene indicating structure stability. As a conclusion, Thr-52 and Gly-55 are important residues for lid 1 stability as their existence helps to retain the geometrical structure of alpha-helix and connecting hinge.

Keyword: Lid engineering; Cold-active lipase; Molecular dynamics simulation; Hydrophobic contacts; Site-directed mutagenesis; Solvent accessibility; Substrate affinity; Thermostability