The effects of one amino acid substitutions at the C-terminal region of thermostable L2 lipase by computational and experimental approach

ABSTRACT

The substitutions of the amino acid at the predetermined critical point at the C-terminal of L2 lipase may increase its thermostability and enzymatic activity, or even otherwise speed up the unfolding of the protein structure. The C-terminal of most proteins is often flexible and disordered. However, some protein functions are directly related to flexibility and play significant role in enzyme reaction. The critical point for mutation of L2 lipase structure was predicted at the position 385 of the L2 sequence, and the best three mutants were determined based on I-Mutant2.0 software. The best three mutants were S385E, S385I and S385V. The effects of the substitution of the amino acids at the critical point were analysed with molecular dynamics simulation by using Yet Another Scientific Artificial Reality Application software. The predicted mutant L2 lipases were found to have lower root mean square deviation value as compared to L2 lipase. It was indicated that all the three mutants had higher compactness in the structure, consequently enhanced the stability. Root mean square fluctuation analysis showed that the flexibility of L2 lipase was reduced by mutations. Purified S385E lipase had an optimum temperature of 80 °C in Tris–HCl pH 8. The highest enzymatic activity of purified S385E lipase was obtained at 80 °C temperature in Tris–HCl pH 8, while for L2 lipase it was at 70 °C in Glycine–NaOH pH 9. The thermal stability of S385V lipase was enhanced as compared to other protein since that the melting point (T m) value was at 85.96 °C. S385I lipase was more thermostable compared to recombinant L2 lipase and other mutants at temperature 60 °C within 16 h preincubation.

Keyword: Flexibility; L2 lipase; Molecular dynamics (MD) simulation; Site-directed mutagenesis; Stability