

Biochemical and biophysical characterisation of a small purified lipase from *Rhizopus oryzae* ZAC3

Zainab A. Ayinla ; Adedeji N. Ademakinwa ; Richard A. Gross ; **Femi K. Agboola**

Abstract

The characteristics of a purified lipase from *Rhizopus oryzae* ZAC3 (RoL-ZAC3) were investigated. RoL-ZAC3, a 15.8 kDa protein, which was optimally active at pH 8 and 55 °C had a half-life of 126 min at 60 °C. The kinetic parameters using *p*-nitrophenylbutyrate as substrate were 0.19 ± 0.02 mM, 126 ± 5.6 U/ml and 122 s^{-1} for K_m , V_{\max} and k_{cat} respectively. RoL-ZAC3 showed stability in methanol and isopropanol with Na^+ enhancing the activity. *p*-nitrophenyloleate and castor oil were the best preferred substrates among the *p*-nitrophenyl esters and vegetable oils tested respectively. About 43% residual activity was observed after incubation for 30 min at 75 °C. Circular dichroism thermal scan showed that the lipase displayed intense negative ellipticities even at high temperature. Perturbation of the tertiary structure with increasing temperature caused the exposure of hydrophobic side chains to the aqueous environment as revealed by tryptophan fluorescence, with a $t-T_m$ of 50 °C. Differential scanning calorimetry analysis showed melting temperature and calorimetric enthalpy of 55.5 °C and 444 kJ/mol respectively. Dynamic light scattering analysis indicated that the lipase was prone to aggregation upon unfolding at high temperature. It can be concluded that RoL-ZAC3 possesses promising potential for numerous biotechnological applications.

Keywords: Lipase ; *Rhizopus oryzae* ; tryptophan fluorescence ; circular dichroism ; calorimetric enthalpy

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