

Enhancement of a protocol purifying T1 lipase through molecular approach

ABSTRACT

T1 Lipase is a thermostable secretory protein of *Geobacillus zalihae* strain previously expressed in a prokaryotic system and purified using three-step purification: affinity 1, affinity 2, and ion exchange chromatography (IEX). This approach is time consuming and offers low purity and recovery yield. In order to enhance the purification strategy of T1 lipase, affinity 2 was removed so that after affinity 1, the cleaved Glutathione S-transferase (GST) and matured T1 lipase could be directly separated through IEX. Therefore, a rational design of GST isoelectric point (pI) was implemented by prediction using ExPASy software in order to enhance the differences of pI values between GST and matured T1 lipase. Site-directed mutagenesis at two locations flanking the downstream region of GST sequences (H215R and G213R) was successfully performed. Double point mutations changed the charge on GST from 6.10 to 6.53. The purified lipase from the new construct GST tag mutant-T1 was successfully purified using two steps of purification with 6,849 U/mg of lipase specific activity, 33% yield, and a 44-fold increase in purification. Hence, the increment of the pI values in the GST tag fusion T1 lipase resulted in a successful direct separation through IEX and lead to successful purification.

Keyword: E. coli; Isoelectric point; Lipase; Purifications; Site-directed mutagenesis