P86

Purification and Characterization of Recombinant Lipase Produced by Refolding System

Sun-Young An, Sang-Wan Kim, Yong-Lark Choi, Young-Su Cho, Woo-Hong Joo[1] and Young-Choon Lee

Division of Biotechnology, Faculty of Natural Resources and Life Science, Dong-A University, Busan 604-714; Dept. of Biology, Changwon National University, Kyungnam 641-773, Korea

We have previously cloned a novel-type bacterial lipase gene which is classified into lipase family I.1 based on the amino acid sequence similarities but into lipase family I.2 based on molecular weight (33 kDa) and active-site motif (GHSQG). When this gene was expressed under T7 promoter using a expression vector, pET29a(+) in *Escherichia coli* (BL21 DE3), recombinant lipase was overproduced in an insoluble form. To obtain an active enzyme, after this protein was solubillized in the presence of 8 M urea, it was purified in a urea-denatured form by ion-exchange chromatography on DEAE-Sepharose and refolded by removing urea in the presence of the Ca^{2+} ion. Gel filtration chromatography indicated that this refolded protein is monomeric. Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) revealed an molecular weight of 33 kDa for the purified protein. rPSL showed relatively broad substrate specificities. The optimum pH and temperature was pH 8.0 and 50℃, respectively. Th enzyme was stable in the range of pH 8-9 and at below 50℃ in the presence of 5 mM $CaCl_2$. Among trhe p-nitrophenyl (PNP) esters tested the best substrate was PNP-myristate with *K*m and *V*max values of 44 μM and 24 nM/min^{-1}.