PURIFICATION AND CHARACTERIZATION OF LIPASE LIP3 PRODUCED IN SUBMERGED FERMENTATION BY *RHIZOPUS ARRHIZUS* HR1

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Abstract

Lipases (E.C. 3.1.1.3) are triacylglycerol acylhydrolases that catalyze the hydrolysis of triacylglicerols to glycerol and free fatty acids and the reverse reaction of esterification, transesterification and interesterification in anhydrous medium. Because of their wide application in different industries lipases are the third most important category of enzymes, after carbohydrolases and proteases. They are ubiquitous enzymes produced by several plants, animals and microorganisms. Microbial lipases have gained special industrial attention because of their high stability in extremes of temperature, pH and organic solvents, and their regio-, chemo- and enantioselectivity. Often, microbial strains synthesize multiple forms of lipase that differ in their properties. Knowledge of the properties and characteristics of lipases allows their proper application. The aim of this study is purification of lipase from *Rhizopus arrhizus* HR1 and characterization of the crude enzyme and one of the purified multiple forms of lipases, designated as Lip3.

The studied industrial *Rhizopus arrhizus* HR1 strain used in this study was provided by Biovet JSC. The culture liquid obtained after submerged cultivation of the strain was used for isolation of lipase. A lipase (Lip3) was purified by a three-step purification protocol: fractional precipitation with 60% polyethylene glycol 400 (PEG 400), ion-exchange chromatography with diethylaminoethanol covalently linked to sepharose (DEAE-Sepharose), and size-exclusion chromatography with Sephadex G100. The main biochemical characteristics of the isolated lipase were studed: influence of pH and temperature on the catalytic ability of the enzyme, as well as the process of its thermal inactivation. The influence of pH was tested in the range of 3 - 11 with 0.05 M phosphate buffer with pH 3 - 6, 0.05 M Tris-HCl buffer with pH 7 - 9 and 0.05 M sodium-carbonate buffer with pH 10 - 11. The influence of temperature on lipase activity was tested in the range of 20 - 50 ^{0}C at pH 9. The activation energy Ea (kJ/mol) of the reaction catalyzed by Lip3 was determined by plotting the linear relationship between ln (v) and 1/T (K) according to the Arrhenius equation. The thermodynamic parameters of temperature inactivation process at 30 and 40 ^{o}C were determined. The experiment was conducted at 30 and 40 ^{0}C for 1 h and pH 5. Lipase stability was investigated in the presence of the metal salts in concentrations 1 mM, 5 mM and 10 mM. The enzyme was incubated in the presence of the salts at room temperature for 30 min. The influence of some organic solvents (methanol, ethanol, 2-propanole, acetone, hexane, DMSO and DMF) in concentrations 10 %, 20 % and 40 % was tested. Lipase activity was measured by p-nitrophenyl palmitate method.

Purified lipase was a glycoprotein with Mm = 188 kDa. Crude lipase had temperature optimum at 30 ^{0}C and pH optima in the range of 7 - 9, while purified Lip3 had highest enzyme activity at 35 ^{0}C and pH 9. Activation energy for both crude enzyme and Lip3 was found to be respectively 20.27 kJ/mol and 39.00 kJ/mol. Thermal stability was also studied and it was found that crude lipase was more stable with $t_{1/2}$ = 620 min at 30 ^{0}C and $t_{1/2}$ = 32 min at 40 ^{0}C, in comparison to Lip3, which displayed $t_{1/2}$ = 26 min at 30 ^{0}C and $t_{1/2}$ = 7 min at 40 ^{0}C. Influence of some metal ions and organic solvents was also examined. Al^{3+} had slightly activating action on crude lipase while Lip3 was activated by Fe^{2+}, Mg^{2+}, Cu^{2+}, Al^{3+} Mn^{2+} and Hg^{2+}. Pb^{2+} and Ce^{2+} were found to be strong inhibitors. Some organic solvents like hexane, dimethyl sulfoxyde (DMSO) and dimethyl formamide (DMF) increased lipase activity while short-chained alcohols and acetone were inhibitors activated by Fe^{2+}, Mg^{2+}, Cu^{2+}, Al^{3+} Mn^{2+} and Hg^{2+}. Pb^{2+} and Ce^{2+} were found to be strong inhibitors.

This information is important from the point of view that lipase from *Rhizopus arrhizus* HR1 can be used for realizing enzymatically catalyzed transesterification reactions.

Key words: *Lipase, Rhizopus arrhizus, Submerged fermentation, Purification, Properties.*