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Isolation, Purification and Characterization of Secondary Structure and Kinetic Study of Lipase from Indian Major Carp, *Catla catla* (Catla)

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Abstract

Lipases are ubiquitous enzymes which catalyze the hydrolysis of fats into fatty acids and glycerol at the water-lipid interface and reversing the reaction in non-aqueous media. Lipases occupy a place of prominence among biocatalysts owing to their novel and multifold applications in oleochemistry, organic synthesis, detergent formulation and nutrition.

Lipase was extracted and isolated from the alimentary canal and digestive gut of *Catla catla* (catla). The tissue was homogenized in a ratio of 1:3 with starting buffer (0.01 M TrisHCl, pH 7.2). The crude extract thus obtained was precipitated using ammonium sulphate (20-80%). Excess salt was removed by Dialysis and the resultant dialysate (Desalted Enzyme) was subjected to DEAE-Cellulose column for Ion Exchange Chromatography at a flow rate of 0.5 ml/min. Elution was carried out by a step gradient of NaCl (100-800 mM) in the starting buffer. Active fractions were pooled as Purified Fraction (PF) and were used for physical characterization of pH, temperature and effect of calcium on the enzyme activity, structural characterization, molecular characterization and for kinetic studies. The Purified Fraction (PF) showed final specific activity of 1438.72 U/mg. The optimum pH was 7.8 and the optimum temperature was found to be 20˚C. Melting Temperature (T_m) value was 42˚C and the activation energy of purified lipase was 34.82 KJ/mol/K. Thermal stability of lipase was found to be at 20˚C. Lipase activity retained up to 3 h of incubation with 10 mM and 20 mM of $CaCl_2$ in starting buffer. This shows that Calcium has enhancing property from the denaturation of enzyme. Michaelis-Menten constant (K_m) of lipase from Indian major carp, catla for the hydrolysis of pNPP was 6.695 mM. Turnover number (k_{cat}) of lipase (catla) was 0.0022 s^{-1}. Catalytic efficiency (k_{cat}/K_m) of lipase was 0.0003412 s^{-1} mM^{-1}. SDS-PAGE of purified lipase (PF) revealed a homogenous single band with molecular mass of 70 kDa. Secondary structural arrangement of α helices and β strands of purified lipase results 48.51% and 9.74%, respectively using Circular Dichroism.

Keywords: Lipase; Ammonium sulfate; Purification; Characterization; Kinetic study

Introduction

The use of enzymes created opportunities for developing a green, sustainable and modern industrial chemistry due to excellent specificity, being atom economic, mild reaction conditions, energy-saving process and simplicity. In particular the use of hydrolytic enzymes such as protease, lipase and amylase in the industrial enzyme market is growing steadily.

Lipases (E.C. 3.1.1.3) can be broadly defined as enzymes that catalyze the hydrolysis of ester bonds in substrates such as vitamin esters, phospholipids, triglycerides (TGs) and cholesteryl esters [1]. Interest in lipases has been increasing in the past few years because of the actual and potential uses of this enzyme in applications like hydrolysis of fats and oils, organic synthesis, modification fats, chemical analysis and flavor enhancement in food production. The isolation and purification of lipases is reported in various models, chiefly microorganisms but there is a paucity of information about the isolation and purification from carps. Therefore, the lipases from the tissues of these aquatic vertebrates have received much less attention than those from microorganisms. Hepatopancreas as digestive organs found in the fishes remain unexplored lipolytic enzyme sources that could have unique characteristics. Fish lipases demonstrate novel activities that have potential industrial applications [2]. Purification of lipase from the Indian Major Carps may also be of benefit for the development in the field of aquaculture. Thus the present study intricacies on the isolation purification and characterization of lipase from the digestive gut of Indian Major carp, *Catla catla* (catla).

Materials and Methods

Culture of fish

Indian major carp *Catla catla* (catla) were collected from local fish market, INA, Delhi and were acclimatized at outdoor conditions for 10 days. Fish were fed with artificial diet containing 40% protein. Artificial diet was prepared by using dried fish powder, wheat flour, cod liver oil and vitamin and mineral premixes [3]. Fishes were kept in fasting condition for 48 h before sampling.

Sampling of tissue

Two fishes were anesthetized with MS 222 (Tricaine methanesulfonate) before sacrifice. Individual fish was dissected; digestive system and associated glands (hepatopancreas) were removed from the body, cleaned, weighted and immediately frozen at -20°C till further use.

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