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Isolation, Identification and 3D Structure of Extracellular Lipase from *Mucor hiemalis* by Gel Electrophoresis

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Abstract: This study was undertaken to locate and identify extracellular lipase from *M. hiemalis* by gel electrophoresis. Lipase activity was shown by blue band. 3 dimensional structure of lipase was also discussed. Active site of lipase is Leu²⁰⁵ and glun³⁴. Surface of enzyme consist of both hydrophobic and hydrophilic amino acid and this help to clear the lipid from blood.

Key words: Lipase, Electrophoresis, 3 dimensional structure

Introduction

Lipolytic enzymes are indispensable for biological turn over of lipids. They are required as digestive enzyme in hydrolysis of triglyceride present in VLDL (very low-density lipoprotein) and Chylomicron. Chylomicron is metabolized in adipose tissue and muscle. Enzyme lipoprotein lipase located on capillary walls, is activated by apolipoprotein (apo) C 11 and then hydrolysed triglyceride and fatty acid. VLDL is a large triglyceride rich particle incorporating apo B, apo C and apo E. After secretion it incorporates more apo C from HDL (high density lipoprotein). In peripheral tissue, triglyceride removed after hydrolysis by lipase then VLDL converted into IDL (intermediate density lipoprotein). Lipoprotein lipase also affects HDL metabolism. Importance of lipase in HDL metabolism has been recognised because of the fact that patient with lipase deficiency show low HDL-cholesterol and high triglyceride concentration. Enzyme plays an important role in controlling cardiovascular diseases by removing lipids from circulation. Besides, it has an important role in diabetes. It is reported that abnormalities in lipid metabolism may be secondary to insulin deficiency. Lipolysis is enhanced and plasma free fatty concentration rise. In the liver free fatty acid converted into acetyl CoA and ketones or are re-esterified to form endogenous triglyceride and incorporated into VLDL. The latter accumulate into plasma because lipoprotein lipase, which is necessary for VLDL catabolism, requires insulin for optimal activity. Lipase also use for deposition and mobilization of fats therefore used as energy reservoirs and in functioning of biological membrane (Stryer, 1992). Purified lipase is in tremendous use in industrial and pharmaceuticals in particular. Lipolytic enzyme also important as analytical and synthetical tools (Arima *et al.*, 1972).

Purpose of study is to locate and identify of extracellular lipase from *Mucor hiemalis* by chromatography and electrophoresis. 3 dimensional structure of lipase was taken by Protein data bank file (PDB file) and its active centre and other sites will be studied by using computer program Rasmol.

Materials and Methods

Strain used was *Mucor hiemalis*:

Fermentation: Fermentation of enzyme was done by using the method of Kuksis (1983). It was performed at 26°C by using the medium consisting by using the medium consisting of 27 g peptone, 18 g potassium dihydrogen phosphate. 0.45 g potassium chloride and 0.45 g magnesium sulfate. 10 ml purified commercial olive oil was used as carbon source. Fermentation in flask was carried out using 10 percent olive oil in gum acacia and autoclaved at 15 lb pressure for 30 minutes.

Enzyme assay: Method was taken from Varley *et al.* (1988). Olive

oil was emulsified in 10 percent gum acacia for 10 minutes with the help of homogeniser. It is used as substrate. For extracellular *hiemalis* lipase, add into a vial, 1 ml of enzyme, 1 ml of substrate, 0.2 ml of 0.1 M calcium chloride and 0.05 M of tris HCl buffer (pH 7.5). Vial was incubated at 37°C for 60 minutes with continuous shaking in Gallenkamp Shaker. After incubation, 3 ml of alcohol and thymophthylin acetone in a ratio of 1:1 was added to stop the rate of reaction. Thymophthylin was used as indicator. End point was marked by the change of colour from milky white to blue. Blank was prepared only when enzyme was added after the addition of alcohol and acetone in ratio of 1:1. A unit of enzyme is that amount of enzyme that would liberate 1 μ M free fatty acid from olive oil.

Salting Out: Salting out was performed by the method of Bollag *et al.* (1996a). Lipase was first concentrated by salting out with ammonium sulphate. In broth filtrate, ammonium sulphate was added to a definite saturated and incubated of 40°C for two hours. Precipitates obtained were separated by centrifugation. These precipitates were dissolved in 20 ml of chilled distilled water.

Chromatography: Bollag *et al.* (1996b) used chromatographic techniques for different purposes like desalting, filtration etc. Desalting was performed by using sephadex G25. Column (2.2 × 42 cm) was packed with gel and washed with 50 ml-distilled water. 15 ml of enzyme sample was loaded. Eluted proteins were obtained and lyophilised.

Electrophoresis: Rod gel electrophoresis was performed according to the technique of Lakshmi and Subramangan (1977). Gel was prepared by mixing the solution of acrylamide, water and lipid substrate in a ratio of 1.2:1:0.1. It was homogenised for 10 minutes until a uniform white emulsion formed. This emulsion was mixed with an equal volume of ammonium per sulphate solution. 2 ml aliquates of the mixture obtained and transferred into glass tubes (7 × 0.6 cm). Another set of gel were prepared in a similar manner but without addition of substrate. Top surface of the gel was layered with distilled water. Tubes were left at room temperature for gelation to occur then the gels were cooled at 40°C for two hours. After pouring separating gel into the tube, 0.1 ml of stacking gel containing 3.75 percent acrylamide was added. After polymerization 0.2 ml of sample gel containing 3.75 percent acrylamide and 200 μ g of sample was loaded. After polymerization rest of tubes were filled with electrode buffer (0.05 M tris HCl, pH 8.3), diluted four times with distilled water. Electrophoresis was carried out at 40°C for 30 minutes (Fig. 1).

Model of lipase: 3 dimensional (3D) structure of lipase consists



Fig. 1: Single band of lipase on agarose gel electrophoresis



Fig. 2: a) Structure of lipase shows active center located at aln³⁴ and leu²⁰⁵ b) Ribbon diagram of lipase shows N and C terminals

of 4673 atoms having 4760 hydrogen bonds. Three-dimensional structure of enzyme was solved at 0.157 Å resolution. Lipase is mainly consist of two chains that is chain A and B. Structure of lipase composed of 5 helices, FL¹ and FL² form a flap covering the active site. Active site is mainly consisting of Leu²⁰⁵ and glun³⁴. N terminal is ala¹ and C terminal is val³²⁰ stabilized by hydrogen bonds. Stability is enhanced by entropic effect of disulphide bond. Structure is predominantly a structure with each of domain is a deformed a bend. Over all tertiary fold is shown by ribbon representation (Fig. 2). It consist of 4-N acetylglucose amine residue at three sites i.e. 2-N linked residue at Asn 364, 1-N

linked residue at Asn 283 and 1-O linked residue at Thr 4. Surface of enzyme consist of both hydrophobic and hydrophilic amino acids. This shows the reactivity of enzyme by which it clear the lipids from blood.

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