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RESEARCH PAPER

Purification and Characterization of a Lipase from *Aspergillus niger* F044

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Abstract: A lipase from *Aspergillus niger* F044 was purified to homogeneity using ammonium sulfate precipitation, dialysis, DEAE-Sepharose Fast Flow anion exchange chromatography, and Sephadex G-75 gel filtration chromatography. This purification protocol resulted in a 73.71-fold purification of lipase with 33.99 % final yield, and the relative molecular weight of the lipase were determined to be approximately 35–40 kD using SDS-PAGE. The optimal pH and temperature for lipolytic activity of the lipase was 7.0 and 45 °C, respectively. It was stable at temperature up to 60 °C and retained 98.70 % of its original activity for 30 min. The stability declined rapidly as soon as the temperature rose over 65 °C. The lipase was highly stable in the pH range from 2.0 to 9.0 for 4 h. Ca^{2+} and Mg^{2+} ions stimulated its lipolytic activity, whereas Mn^{2+}, Fe^{2+}, and Zn^{2+} ions caused inhibition. The lipase was also relatively stable in methanol, 2-propanol, and methyl acetate at a final concentration of 40 % (*V*/*V*) for 24 h. The substrate exhibited a broad specificity toward various oils. The values of K_m and V_{max} calculated from the Lineweaver–Burk plot using *p*-nitrophenyl palmitate as hydrolysis substrate were 7.37 mmol/L and 25.91 μmol·min^{-1}·mg^{-1}, respectively. The N-terminal amino acid sequence of the lipase was Ser/Glu/His-Val-Ser-Thr-Ser-Thr-Leu-Asp-Glu-Leu-Gln-Leu-Phe-Ala-Gln, which is highly homogeneous with that of lipase.

Key Words: *Aspergillus niger*; lipase; purification; enzyme characterization

Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) catalyze the hydrolysis or the synthesis of esters formed from glycerol and long-chain fatty acids at the interface between the insoluble substrate and water. These reactions usually proceed with exquisite chemoselectivity, regioselectivity and stereoselectivity, making lipases an important group of biocatalysts in biotechnology. Lipases are widely used in food processing, in production of biodegradable polymers, in synthesis of fine chemicals, in production of biodiesel, as additive to detergents, in leather industry, in pulp and paper manufacture, etc[1].

A. niger lipase displays positional selectivity toward the 1- and 3-positions of the glycerol moiety[2–4], substrate preference for medium-chain length of fatty acids, and is considered GRAS (Generally Regarded As Safe) by the Food and Drug Administration of the United States of America, which make it a conventional food additive in the food processing industry[5]. Recently, the *A. niger* lipase has been applied to the acetylation of cellulose[6] and the degradation of ochratoxin A[7], which resulted in expansion of its application fields.

The enzyme characterization of *A. niger* lipases that originated from different strains varied considerably[2,8,9], and some *A. niger* strains produced lipase isoenzymes with different characterization[10,11]. A lipase overproduction strain was isolated from oil-stained soil samples, and the organism was identified and named as *A. niger* F044 in the authors' laboratory[12]. The purification and enzyme characterization of

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the lipase produced by *A. niger* F044 was investigated in this article.

1 Materials and methods

1.1 Microorganism

A lipase overproduction strain was isolated in the authors' laboratory from oil-stained soil samples collected from an oil processing plant in Wuhan, China. Based on the morphological characteristics (colony and mycelium), phylogenetic analysis of the ITS1-5.8S rRNA gene-ITS4 sequences and the target for the restriction endonuclease *Rsa*I that existed in the ITS1-5.8S rRNA gene-ITS4 sequences, the organism was identified and named as *A. niger* F044 according to the taxonomy standard of the *A. niger* aggregate[12–14].

1.2 Methods

1.2.1 Lipase production: The spore suspension of *A. niger* F044 was prepared in sterile distilled water and used as inoculum. The lipase production medium contained 1.5 % maltose (*W*/*V*), 1.25 % beef extract, 0.1 % $(NH_4)_2SO_4$, 0.2 % K_2HPO_4, 0.01 % $MgSO_4.7H_2O$, and emulsification of olive oil 4 % (*V*/*V*). The spore suspension was inoculated into a series of 500-mL shake flasks containing 50 mL of lipase production medium and incubated by reciprocal shaking at 250 r/min and 28 °C for 80 h. Cultures were filtered and the filtrate containing the extracellular lipase was centrifuged at 8 000 r/min and 4 °C for 15 min. The cell-free supernatant was used as crude lipase source for purification.

1.2.2 Ammonium sulfate precipitation: Well-ground ammonium sulfate was slowly added to 250 mL of the cell-free supernatant to 60 % saturation under stirring conditions at 4 °C. Precipitation was allowed for 3–4 h at 4 °C. After centrifugation, the precipitate was collected and dissolved again in 50 mL of 20 mmol/L His-HCl buffer (pH 6.5). The preparation was dialyzed against the same buffer overnight at 4 °C.

1.2.3 Anion exchange chromatography: The desalted lipase solution was centrifuged at 12 000 r/min and 4 °C for 20 min, and the supernatant was loaded on a DEAE-Sepharose Fast Flow column (2.0 cm × 20 cm) pre-equilibrated with 20 mmol/L of His-HCl (pH 6.5). After the column was rinsed with 150 mL of equilibration buffer, the lipase was eluted with 250 mL of 0–1 mol/L NaCl step gradients in the same buffer with a flow rate of 20 $mL \cdot h^{-1}$. The active fractions were pooled and concentrated in a vacuum evaporator.

1.2.4 Gel filtration chromatography: The freeze-dried lipase sample was dissolved in 5 mL of 50 mmol/L Tris-HCl (pH 7.5) and then applied to a Sephadex G-75 column (1.0 cm × 60 cm) which was pre-equilibrated with 50 mmol/L of Tris-HCl (pH 7.5). The lipase was eluted with the same buffer at a flow rate of 20 $mL \cdot h^{-1}$. The active fractions were pooled, concentrated, and the purity was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis.

The lipase activity was detected using rapid qualitative assay method on olive oil-rhodamine B agar plates during purification.

1.2.5 Determination of protein concentration: The protein concentration of the lipase samples was determined using the method of Bradford[15], with bovine serum albumin as the standard.

1.2.6 Assays for lipase activity: Lipase activity was determined using alkali titration method as described by Saxena[16], using spectrophotometric assay method as described by Kordel[17], and using rapid qualitative assay method on olive oil-rhodamine B agar plates as described by Hiol[18]. The alkali titration method was used in the whole experiment unless stated otherwise.

All reactions were carried out at 45 °C and 50 mmol/L of Tris-HCl buffer (pH 7.5), 2 mmol/L of $CaCl_2$ unless stated otherwise. One unit of lipase activity was defined as 1 μmol of fatty acid or 1 μmol of *p*-nitrophenol liberated from olive oil or pNPP per min by 1.0 mL of lipase under the standard assay conditions.

1.2.7 Determination of the relative molecular mass and the purity of the purified lipase: The relative molecular mass of the purified lipase was determined using SDS-PAGE gel electrophoresis on a 12 % separating gel[19]. The purity of the purified lipase was assayed by high-performance liquid chromatography (HPLC).

2 Results and analysis

2.1 Purification of *A. niger* F044 lipase

The lipase was finally purified to 73.71-fold, starting from the culture filtrate with a yield of 34 % by ammonium sulfate precipitation, DEAE-Sepharose fast flow column chromatography, and Sephadex G-75 gel filtration chromatography. The purification efficiency using anion exchange chromatography was the, highest and 50-fold increase in specific activity was obtained only using this step. The purification results are summarized in Table 1. The purified lipase was homogenous when tested using SDS-PAGE and showed only a single band (Fig. 1). In view of the monomeric form of all the known *A. niger* lipase[2,8,9], the relative molecular mass of the *A. niger* F044 lipase was approximately 35 kD–40 kD, which was the same as those of *A. niger* lipase reported previously[2,8,9]. The specific activity of the purified *A. niger* F044 lipase was much higher than that of other *A. niger* lipase reported previously, 170 u/mg[8] and 729 u/mg[20].

2.2 Effect of metal ions and EDTA on the *A. niger* F044 lipase activity

The purified lipase solution was dialyzed against 0.05 mol/L of Tris-HCl buffer (pH 7.5) overnight at 4 °C, and then the various metal ions and EDTA were added into the dialyzed lipase solution to a attain a final concentration of 2