PREPARATION, PURIFICATION AND PROPERTIES OF LIPASE FROM HEPATOPANCREAS OF TRA (*PANGASIUS*) CATFISH

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ABSTRACT: *Lipase from the hepatopancreas of Tra (Pangasius) catfish was purified by ammonium sulfate fractionation, followed by ion-exhange chromatography on DEAE Cellulose and gel filtration Sephadex G-75. The preparation was homogeneous on polyacrylamide disc gel electrophoresis. The specific activity of the purified enzyme was 37.95 times higher than that of the crude extract. The enzyme showed a molecular weight of 57000 Da. The pH and temperature optima of purified lipase were 8 and 50^0C respectively. Enzyme activity was enhanced by Ca^{2+} but inhibited by heavy metals Zn^{2+}, Cd^{2+}, Mg^{2+}.*

Keywords*: lipase, hepatopancreas, purification, properties.*

1. INTRODUCTION

Lipases (triacylglycerol acylhydrolases E.C. 3.1.1.3) are enzymes that catalyze the hydrolysis of triacylglycerols at the oil-water interface to release glycerol and free fatty acids. Lipases are finding increasing uses as food and other industrial processing aids, thus there is growing interest in discovering new sources of these enzymes with appropriate characteristics to suit particular applications. The few lipases that have been studied from fish and other aquatic animals include lipases from the leopard shark (Patton et al., 1977), Atlantic cod (Lie and Lambersten, 1985; Gjellesvik et al., 1992), dog fish (Raso and Hultin, 1988), sardine (Mukundan et al., 1985), anchovy, striped bas and salmon (Lager et al., 1977). The present paper focuses on the purification of Tra (*Pangasius*) pancreatic lipase and the effects of temperature, pH and metal ions on the enzyme activity.

2. MATERIALS AND METHODS

2.1. Hepatopancreas collections

All hepatopancreases were collected from a local slaughterhouse (Mekong Delta region, Viet Nam). Tra (*Pangasius*) catfish hepatopancreases of different species were removed immediately after death and kept at −20 ◦C.

2.2. Preparation of crude enzyme

After trimming the excess fat , the Tra catfish hepatopancreases were cut into small pieces (1–2 cm^2) and ground mechanically twice for 60s at 5◦C . The mixture was suspended in buffer A: 50mM Tris–HCl, pH 8 with mixing ratio 1:2 (w/v) of mixture to buffer and stirred

with a magnetic bar for 60min at 5 ◦C, and then centrifuged for 20min at 6,000 rpm. After removing insoluble particles, crude enzyme was obtained.

2.3. Purification of hepatopancreas lipase

2.3.1. Ammonium sulfate precipitation

Crude enzyme extraction was brought to 60% saturation with solid ammonium sulfate under stirring conditions and maintained for 60 min at 5^0C. After centrifugation (20 min at 6,000 rpm), the pellet was resuspended in minimum volume of buffer A.

2.3.2. Dialysis

After ammonium sulfate precipitation, the enzyme was dialyzed against distilled water for 12 h and against the buffer (10mM Tris-HCl pH8) for 12 h at 5^0C.

2.3.3. Anion exchange chromatography

After dialysis, the enzyme solution was loaded on a column (2.0 x 15 cm) of diethylaminoethyl (DEAE) cellulose equilibrated with buffer A. Under these conditions, the enzyme did not adsorb on the cationic support and was eluted during a washing by the same buffer A.

2.3.4. Filtration on Sephadex G-75

Active fractions eluted from DEAE cellulose were pooled and loaded on a gel filtration Sephadex G-75 column (1.2 x 60 cm) equilibrated with buffer A. Elution of lipase was performed with buffer A at 25 ml/h

2.3. Lipase assay and protein estimation

The lipase activity was measured titrimetrically at pH 8 and 37 ^{0}C with pH-Stat using olive oil emulsion. One lipase unit corresponds to 1 µmol of fatty acid released per minute. (Mukunda et al., 1985)

Protein content of the enzyme was determined by the method of Lowry et al. (1951) using BSA as standard.

Analytical polyacrylamide gel electrophoresis of proteins in the presence of sodium dodecyl sulfate (SDS-PAGE) was performed by the method of Laemmli et al., (1970).

2.4. The affects of pH and temperature

The enzyme was also studied for the effect of pH 6.0-11.0 and incubation temperature (35-70^{o}C) on enzyme activities. The activities were reported as relative activities compared with the initial enzyme activities.

3. RESULTS AND DISCUSSION

3.1. The level of lipase activities of crude enzyme

The specific lipase activities from the hepatopancreas of Tra catfish (18.44± 1.22 U/mg protein) was higher than that from the hepatopancreas of red sea bream (3.81 U/mg protein) (N.lijima et al. 1998), Tilapia Oreochromis Niloticus (48.5 mU/mg protein) (Rungkan Klahan et al. 2009) but lower than that from caecal mass of Pacific bluefin tuna Thunnus orientalis (27.5 ± 4.6 U /mg protein) (A.M. de la Parra et al. 2007).

3.2. Purification of hepatopancreas lipase of Tra catfish

Table 1. Summary of purification process of hepatopancreas lipase of Tra catfish

Stages	Total protein (mg)	Total activity (units)	Specific activity (units/mg)	Purification (fold)	Yield (%)
Crude enzyme*	202.5	2719.8	13.431	1.0	100
Precipitation	82.35	2493.45	30.279	2.25	91.68
Dialysis	56.7	2293.2	40.444	3.01	84.32
DEAE-cellulose chromatography	4.59	1735	378.288	28.17	63.79
Gel-filtration on Sephadex-G75	2.14	1090	509.714	37.95	40.08

Crude enzyme based on 30 g fresh hepatopancreas of Tra catfish. * Data used to plot figure are average values of duplicate results for experiments

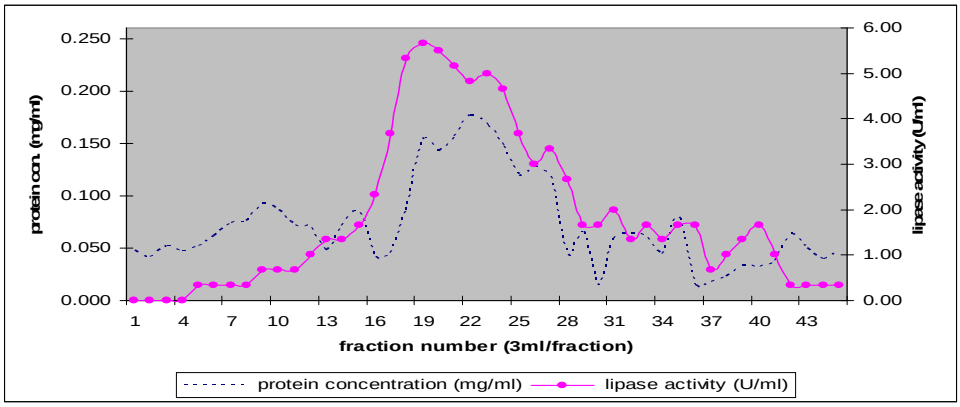

Fig.1. Ion-exchange chromatography of hepatopancreas lipase from Tra catfish on DEAE-cellulose

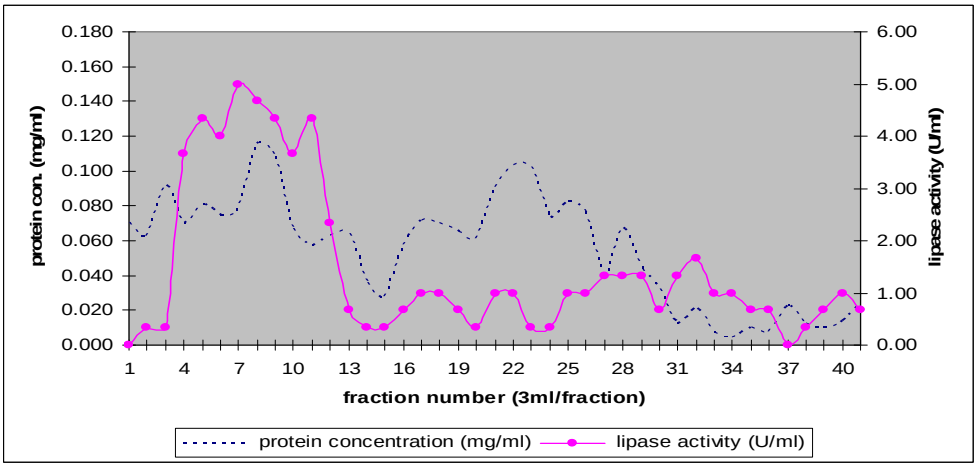

Fig. 2. Gel filtration of hepatopancreas lipase from Tra catfish on Sephadex-75

3.3. Determination of Molecular Weight (MW) of hepatopancreas lipase

The MW of hepatopancreas lipase of Tra catfish was determined by SDS-PAGE using marker proteins was found to be 57 KDa. Naci Degerli et al., (2001) reported the lipase from Cyprinion macrostomus Heckel was 51 KDa and N.Iijima et al., (1988) reported the lipase from red sea beam was 64 KDa. (see Fig. 3)

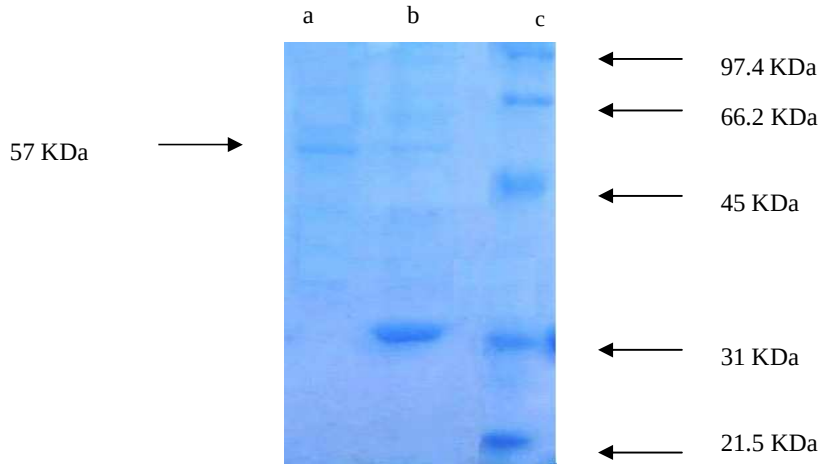

Fig. 3. SDS–polyacrylamide gel electrophoresis (PAGE). Line c: MWM, line b: fractions from DEAE-cellulose chromatography, line a: fractions from Sephadex G-75

3.4. Characterization of hepatopancreas lipase of Tra catfish

3.4.1. Effects of temperature on the activity of lipase

The effects of temperature on the activity of lipase were examined in the range of 35 to 70^0C. In Fig.3 the activity of lipase was increased gradually with rise in temperature and the maximum activity was observed at 50^0C. These results were similar to those reported for lipase from Solea solea at 50°C (Clark et al. 1987) which is slightly lower comparing to grey mullet lipase (55°C) (Aryee et al., 2007) and slightly higher than that of sardine and cod (37^0C) (Gjellesvik et al., 1992; Raso and Hultin, 1988). Data used to plot the figure are average values of duplicate results for experiments.

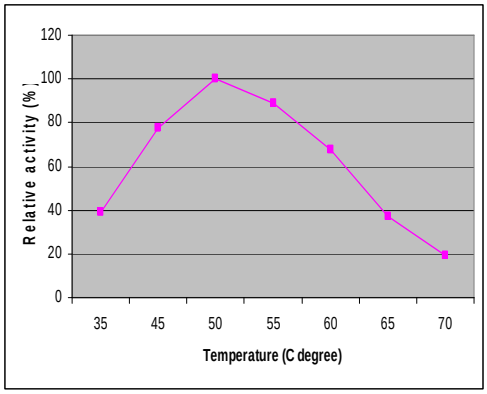

Fig. 3. Effects of temperature on the activity of lipase

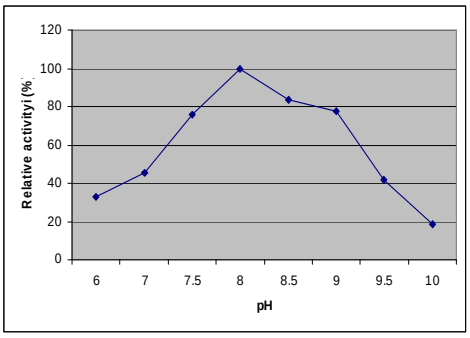

Fig.4. Effect of pH on the activity of lipase

3.4.2. Effect of pH on the activity of lipase

Results revealed that the lipase activity had an optimal pH 8 (Fig. 4). The optimum pH 7~9 for lipase activities were reported for fish and other sources (Prasertsan et al., 2001; Natalia et al., 2004; Gjellesvik et al., 1992). M. K. Mukundan et al. (2006) reported similar results with oil sardine (*Sardinella longiceps linnaeus*) hepatopancreas lipase activity at an optimal pH of 8. Gjellesvik et al. (1989) reported cod lipase activity at an optimal pH of 8.25. while Sheng et al. (2006) reported the maximal activity of lipase from the intestines of hybrid juvenile tilapia (*Oreochromis niloticus* × *Oreochromis aureus*) at a pH level between 6.0 and 9.0. Data used to plot the figure are average values of duplicate results for experiments.

3.3.3. Effect of metal ions on the activity of lipase

Understanding the role of lipase inhibitors may provide a better perceptive of their mechanism of action (Marguet et al., 1994) and successful identification of potent and specific inhibitors have resulted in their application in certain treatments (Bray, 2000; Kotsovolou et al., 2007). In this study, the enzyme was incubated with varios compounds and relative activity was mesured after 30 min. From table 2, it is evident that activity of Tra catfish lipase was inhibited by heavy metals such as Zn^{2+}, Cu^{2+} and Cd^{2+}. Calcium ions carry out a distinct role in the lipase action. Like pancreatic lipase (Liu et al., 1973) Tra catfish lipase was stimulated in the presence of calcium ions. The primiry role of Ca^{2+} seems to be to remove the released fatty acid as its calcium salt.

EDTA, 0.001 activated the lipase by 87%, similar to the results of Lima et al., (2004).

Table 2. Effect of various reagents on the activity of Tra catfish lipase

Reagents	Relative activity (%)
None	100
KCl	108
$CaCl_2$	115
$MgCl_2$	95
$ZnCl_2$	57
$CdCl_2$	38
EDTA	87

4. CONCLUSION

A method for the preparation and purification of a lipase from Tra catfish (Pangasius), is described. The pure enzyme has a molecular weight of 57 Kda. The optimum pH and temperature of hepatopancreas lipase are at 8 and 50^0C, respectively. Lipase activity was stimulated by Ca2+ and inhibited by Mg^{2+}, Zn^{2+}, Cd^{2+}. Further studies were needed to determine its performance in the hydrolysis of unsaturated fish oil.

NGHIÊN CỨU THU NHẬN, TINH SẠCH VÀ XÁC ĐỊNH TÍNH CHẤT CỦA ENZYM LIPASE TỪ GAN TỤY CÁ TRA (*PANGASIUS*)

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TÓM TẮT: *Lipase từ gan tụy cá Tra (Pangasius) được tinh sạch bằng phương pháp kết tủa phân đoạn với muối ammonium sulfate, sau đó qua sắc ký trao đổi ion trên cột DEAE Cellulose và qua sắc ký lọc gel Sephadex G-75. Enzym lipase thu nhận được kiểm tra độ tinh sạch bằng phương pháp điện di trên gel polyacrylamide và xác định phân tử lượng là 57000 Da. Hoạt tính riêng của lipase tinh sạch cao gấp 37,95 lần so với hoạt tính lipase trong dịch trích ly thô. pH tối thích của lipase tinh sạch là 8 và nhiệt độ tối thích là 50^0C. Hoạt tính lipase cao hơn khi có mặt ion Ca^{2+} , ngược lại lipase bị kìm hãm khi có mặt các ion kim loại Zn^{2+},Cd^{2+}, Mg^{2+}.*

Từ khóa*: lipase, gan tụy, tinh sạch, tính chất.*

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