CHARACTERIZATION AND PARTIAL PURIFICATION OF AN INTESTINAL LIPASE

G. Serrero, R. Négrel, and G. Ailhaud
Centre de Biochimie - Faculté des Sciences - Parc Valrose
06034 - NICE - France

Received April 28,1975

SUMMARY An intestinal lipase has been characterized in different specie, including man. A bacterial origin for the rat enzyme and a pancreatic origin for the pig or the human enzyme have been excluded by the use respectively of germ-free rats and of immunosera directed against pig or human pancreatic lipases. The pig enzyme has been purified 242 fold; it is mainly active against short- and medium-chain triglycerides. A role in the absorption of neutral lipids, mainly in pathological situations, is discussed.

Different enzymes of the small intestine catalyze the hydrolysis of carboxylic esters (1). While a lipase from pig intestinal mucosa was described as equally active on mono-, di-, and triglycerides (2), another lipase mainly active on monoglycerides was found in rat intestine and partially purified from chicken mucosa (3,4). In both cases a pancreatic and/or a bacterial origin was not directly excluded for these activities. A lipase, active at acidic pH on short- and medium-chain triglycerides, likely from lingual origin (5), has been found in the stomach of human (6).

It is known that patients with a defect in a general exocrine pancreatic function (7) or with a specific defect involving pancreatic lipase (8,9), will absorb efficiently short- and medium-chain triglycerides. In the present work a lipase, localized in the mucosal cells from intestine of different specie including man, is shown to be different from gastric and pancreatic lipases and not to be of bacterial origin. After a 242 fold purification, the pig intestinal lipase appears to be mainly active against short- and medium-chain triglycerides.

MATERIAL AND METHODS Intestinal cells obtained after careful washes and scraping of the mucosa were homogeneized as descri-

bed in Table I; under these conditions the activity due to pancreatic lipase represents respectively 0%, 3% and 0% of the total lipase activity present in the intestinal cells of human, male Wistar rat (180 to 230 g) and pig.

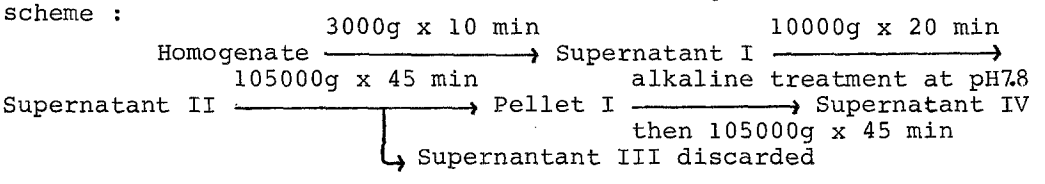
Human biopsies of jejunum and human ileum were obtained through the courtesy of Prof. Delmont and associates (Centre de Gastroentérologie - Université de Nice).

Two different assays of initial rates of hydrolysis were used for intestinal lipase : first a titrimetric assay with tributyrin (at pH 8.3) or triolein (at pH 8.5) as substrates (10,11) and referred as to triglyceride hydrolase activity (E.C.3.1.1.3.), second a spectrophotometric assay with palmityl-CoA as substrate and referred as to thiolesterase activity (E.C.3.1.2.2.). In this last assay the medium contained (in millimolar concentrations; final volume 0.9 ml) : Tris-Cl buffer pH 7.8, 33; NaCl, 150; 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), 0.83 and palmityl -CoA, 0.08. The hydrolysis was followed at 412 nm for at least 2 min. and good proportionnality up to 20 mU per assay was observed. p-nitrophenyl acetate hydrolase was determined by omitting DTNB and replacing palmityl-CoA by 0.5 mM p-nitrophenylacetate. All specific activities are expressed in units (µmoles/min)or milliunits (nmoles/min) per mg of protein (12).

A typical purification of the pig enzyme was as followed :

15 jejunums of pig intestine were removed and thoroughly washed with cold 10 mM Tris-Cl buffer pH 7.3 containing 154 mM NaCl and 10 mM $MgCl_2$. After gentle scraping of the mucosa, the cells were homogeneized at 4°C in 4 volumes (w/w) of 10 mM Tris-Cl buffer pH 7.3 containing 1 mM Ca Cl_2, 10 mM $MgCl_2$, 0.25 M Sucrose and 1 mM DFP.

The starting material was obtained through the following scheme :



Supernatant IV (1400 ml; 1.67 g of proteins) was dialyzed against 30 volumes of 10 mM Tris-Cl buffer pH 7.3 and then placed on a DEAE-cellulose column (3.5 x 30 cm) equilibrated with the same buffer. The elution was performed with 3000 ml of a linear gradient from 0 to 0.5 M NaCl in the same Tris-Cl buffer as above. The activity emerged between 0.1 M and 0.3 M with a peak at 0.2 M. The active fractions were pooled, dialyzed and concentrated batchwise on a second DEAE-cellulose column (2.5 x 5 cm). The concentrated fraction (25 ml, 137 mg) was precipitated at 0.5 saturation with ammonium sulfate. The pellet thus obtained (77 mg) was dissolved in 10 mM Tris-Cl buffer pH 7.3 and put onto a column of Sephadex G-200 (3 x 91 cm) equilibrated with 0.2 M NaCl buffered as above. The active fractions (130 ml; 23 mg) were pooled, dialyzed as usual and further purified on a second DEAE-cellulose column (0.9 x 12 cm) equilibrated with 10 mM Tris-Cl buffer pH 8.5, using a linear gradient from 0 to 0.3 M NaCl (Total volume : 400 ml). The activity emerged as a symetrical peak between 0.06 M and 0.14 M with a maximum at 0.1 M; the pooled fractions were dialyzed against 10 mM Tris-Cl buffer pH 7.3 and stored at 0°C.

Polyacrylamide gel electrophoresis was performed using the Tris-glycine system number 1 according to Maurer (13).

CoA was obtained from P-L Laboratories and the acyl-CoA synthetized according to Ailhaud et al. (14). The different triglycerides were provided by Fluka as well as DFP and N-ethylma-

SPECIES	SPECIFIC ACTIVITIES OF THE HOMOGENATE (mU/mg) WITH		
	TRIBUTYRIN	TRIOLEIN	PALMITYL-CoA
CHICKEN	1500	131	19.5
GUINEA-PIG	1110	206	11.2
CONVENTIONAL RATS	5500	-	22.3
GERM-FREE RATS	5950	-	21.5
RABBIT			
JEJUNUM	3480	90	31.9
ILEUM	2940	218	30.4
PIG			
DUODENUM	1400	850	32.5
JEJUNUM	1900	1130	55.2
ILEUM	-	570	55
MAN			
JEJUNUM	1786	585	10
ILEUM	2080	-	34.2

Table I : Intestinal lipase activity in different specie
The homogenates were obtained after thorough washing of the intestine with cold 0.154 M NaCl.
After gentle scraping of the mucosa, the cells were homogeneized in 10 mM Tris-Cl buffer pH 7.3 containing 1 mM $CaCl_2$, 10 mM $MgCl_2$ and 0.25 M Sucrose and filtered through gauze to remove mucus.

leimide. Iodoacetamide and E_{600} were purchased from Sigma, mono- and diglycerides from Hormel. All other chemicals were obtained from Boehringer. Glyceryltri (1-^{14}C octanoate) was a product of New-England Nuclear.

Immunosera directed against homogeneous pig pancreatic lipase (kindly supplied by Dr. M. Charles, CBM-CNRS, Marseille) and against human and rat pancreatic juices (obtained through the courtesy of Dr. C. Figarella, INSERM, Marseille) were used after precipitation of the immunoglobulin fraction between 0 and 0.4 saturation in ammonium sulfate and dialysis against 20 mM phosphate buffer pH 7.2 containing 150 mM NaCl.

RESULTS Table I shows that the homogenates of all specie including man, contain an hydrolytic activity against tributyrin and/or triolein as well as against palmityl-CoA used as substrates. It was shown with pig intestine (see below) that a single enzyme is active on both substrates.

Of interest is the fact that similar levels were obtained with germ-free rats as compare to conventional rats, which indicates that the activity is not of bacterial origin in this species. As shown for rabbit, pig and man, it is present in the different parts of the intestine. The activity ratio against tributyrin versus palmityl-CoA varies approximately from 25 for the pig to 300 for the rat.

In Table II are given the results of a purification, using Supernatant IV as starting material. Although the starting material only contained a small percentage of the total units present in the homogenate, it was chosen for purification since the use of Supernatant III (which contained variable but up to 59% of the total units) led to rather poor purification. An overall purification of 242 fold was obtained. From the diagrams of Fig. 1 it is apparent that both triglyceride hydrolase and thiolesterase activities are due to the same enzyme. Moreover the ratio values of triglyceride hydrolase versus thiolesterase remain very similar along the purification procedure (last column of Table II), which suggests the absence in the pig homogenate of other hydrolytic activities using triglycerides and/or acyl-CoA as substrates. However the heterogeneity of the 154 fold purified fraction (fraction V) is still obvious, as well as that of fraction VI (not shown). Gel filtration on Sephadex G-200 gave an apparent molecular weight of 70 000 against 48 000 for pancreatic lipase (15).

The curves of immunoprecipitation are presented in Fig. 2.

FRACTION NUMBER	STEP	TOTAL PROTEINS (mg)	TOTAL THIOLESTERASE ACTIVITY (U)	SP. ACT. (U/mg)	YIELD (%)	PURIFICATION (fold)	TRIBUTYRIN activity / PALMITYL-CoA activity
I	HOMOGENATE	76 000	4 452	0.058			22.4
II	SUPERNATANT IV	1 670	388	0.234	8.7	4.2	24
III	DEAE-CELLULOSE at pH 7.3	137	224	1.64	5.3	28	32
IV	AMMONIUM SULFATE PRECIPITATION	77	209	2.7	4.7	47	22
V	SEPHADEX G-200	23	207	9.0	4.65	154	31
VI	DEAE-CELLULOSE at pH 8.5	8.9	125	14.1	2.8	242	30

Table II: Purification of pig intestine lipase
(see Material and Methods for details)

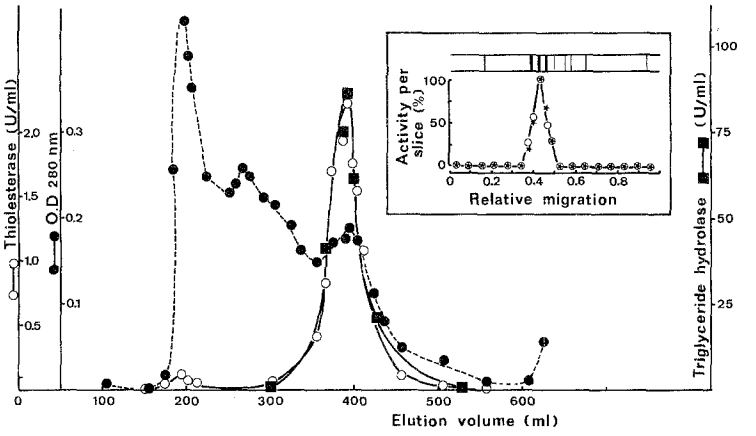


Fig. 1 : Gel filtration of pig intestinal lipase on Sephadex G-200 (see Material and Methods for details) The triglyceride hydrolase was measured with tributyrin as substrate. The inset represents the activity per slice of polyacrylamide gel (2 mm thickness). O : activity measured with glyceryltri ([1 - ^{14}C] octanoate) as substrate; ● : activity measured with palmityl-CoA as substrate. After cutting of the gel, slices were incubated for 48 hours at 4°C in 10 mM Tris-Cl buffer (pH 7.3) and the different supernatants assayed for activity. The 100% value corresponds to the band with an R_f of 0.43.

The absence of any cross-reaction between purified pig intestinal lipase and the immunoserum directed against homogeneous pig pancreatic enzyme clearly indicates the non-identity between intestinal and pancreatic lipases; in the presence of intestinal lipase, the precipitation of pancreatic lipase is normal (curve B). With an excess of immunoserum (16 lipase units per 0.1 ml of immunoserum), a similar lack of precipitation was observed with homogenates prepared from isolated pig intestinal cells (not shown).

Moreover identical results were found when comparing intestinal and pancreatic lipases from rat with an immunoserum directed against rat pancreatic juice (V. Fernandez - Lopez et al.; unpublished experiments).

The absence of identity between intestinal and pancreatic lipases is substantiated by the rates of desactivation at pH 6.2 (half-life of 27 min. and 68 min. respectively) as well as by

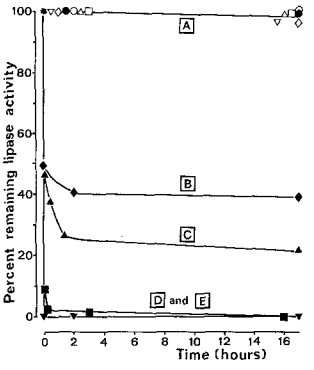


Fig. 2 : Non-identity between intestinal and pancreatic lipases from pig 25 units of hog intestinal lipase (○ and ●), 140 units (△ and ▲), 70 units (□ and ■), 35 units (▽ and ▼) of pig pancreatic lipase and a mixture of 25 units of intestinal and 35 units of pancreatic lipases (◇ and ◆) were incubated in 10 mM potassium phosphate buffer pH 7.3 containing 0.15 M NaCl in the presence of 0.1 ml of rabbit control serum (open symbols) or 0.1 ml of rabbit serum directed against homogeneous hog pancreatic lipase (solid symbols). At the time indicated aliquots were withdrawn and directly assayed for tributyrinase activity; the values obtained at zero time in the control experiments were taken as 100%. The equivalence point was found to be 0.1 ml of immunoserum per 100 units of pig pancreatic lipase.

---

the inhibition with E_{600} under conditions where the pancreatic lipase remains fully active (16). Inhibition of intestinal lipase by E_{600} occurs through a pseudo-first order rate kinetic (half-life at 30°C and pH 8.0 of 130 min. and 10 min. at an inhibitor concentration of 0.1 mM and 1 mM respectively). Iodoacetamide, N-ethyl-maleimide, DFP, FNa and Deoxycholate (1 mM final concentration) had no effect.

An optimum pH was found at pH 7.2 and at pH 8.2 when using palmityl-CoA and tributyrin as substrate respectively, very different from the optimum pH of activity around 5 found for gastric and lingual lipases. The substrate specificity is given in Table III. Although the enzyme is active on long chain fatty acyl-thioesters above C_{12}, it catalyzes the hydrolysis of short- and medium-chain triglycerides preferentially. Both insoluble triesters such as triolein and soluble monoesters such as ethyl

SUBSTRATES		RELATIVE HYDROLYSIS RATE
ACYL-CoA	FINAL CONCENTRATION	
C_2 TO C_8	0.08 mM	0
MALONYL-CoA	"	2.9
LAURYL-CoA	"	10
MYRISTYL-CoA	"	12.6
PALMITYL-CoA	"	14.2
OLEYL-CoA	"	12.2
TRIGLYCERIDES		
TRIACETIN SOLUBLE	250 mM	36
" EMULSIFIED	328 mM	36
TRIBUTYRIN	32 mM	240
TRIHEXANOIN	32 mM	238
TRIOCTANOIN	32 mM	173
TRIOLEIN	32 mM	100

SUBSTRATES		RELATIVE HYDROLYSIS RATE
MONOGLYCERIDES		
1-MONOOLEIN	32 mM	16
MONOESTERS		
ETHYLACETATE SOLUBLE	680 mM	13.7
" " EMULSIFIED	3.4 M	12.8
METHYLCAPRYLATE SOLUBLE	4 mM	57
" " EMULSIFIED	32 mM	13
METHYLLINOLEATE	32 mM	10.8
P-NITROPHENYL ACETATE	0.5 mM	0
MISCELLANEOUS		
PHOSPHOLIPIDS (FROM EGG YOLK)	41 g/L	4.5
TWEEN 20	67 g/L	0

acetate are cleaved by the purified enzyme. A comparison of the positional specificity between pancreatic and intestinal lipases from pig is given in Table IV. Both enzymes are active on 1-monoolein and inactive on 2-monoolein. However the activity toward 1-monoolein is greater for the intestinal lipase than for the pancreatic enzyme (approximately 30 fold) since 400 Units of pancreatic lipase and 31 Units of intestinal lipase (as determined with tributyrin as substrate) were used respectively in the assays.

DISCUSSION The levels of intestinal lipase activity are in the same order of magnitude for the different specie (from 1100 to 6000 mU/mg with tributyrin as substrate).

In pig the experiments of immunoprecipitation, as well as those of desactivation at acidic pH and of inhibition by E_{600}, bring direct evidence that the intestinal lipase is different from the pancreatic enzyme. Moreover the studies of substrate and positional specificities (Tables III and IV) allow to differentiate the hitherto described lipase from that described by Di Nella et al. (2) on crude homogenates, which is equally active on mono-, di- and triglycerides.

The intestinal lipase is concentrated in the villus tip cells which are very sensitive to spontaneous lysis with subsequent loss of activity from the cells and has been partially purified from human intestine (G. Serrero et al., unpublished work). Although present in the absorptive cells of intestine, the role of this enzyme is unclear. A possible role in human, if any, could be an involvment in the absorption of neutral lipids in patients affected by pancreatic deficiencies of different origins (7-9). It is known for instance that infants with congenital absence of pancreatic lipase absorb 30 to 50%

Table III: Substrate specificity of purified pig intestinal lipase
The intestinal rates of hydrolysis are relative to that of triolein taken as 100% (21800mU/mg).
The hydrolysis of acyl-CoA was followed at pH 7.8 and 37°C, and that of the different esters at pH 8.5 and 25°C. Triglycerides (triacetin excepted) and emulsified monoesters were assayed in the presence of Methocell (11), soluble monoesters, phospholipids and Tween 20 in the absence of detergent. The hydrolysis of 1-monoolein was followed titrimetrically at pH 8.5 and 25°C in the presence of (final concentrations) : deoxycholate 0.75%; $CaCl_2$, 0.4 mM and NaCl, 150 mM.

SUBSTRATE	ENZYME	INCUBATION TIME (MIN.)			
		2	5	10	15
1-MONOOLEIN	PANCREATIC LIPASE	4.4	6.7	12.5	25
	INTESTINAL LIPASE	5.5	17	25	37.5
2-MONOOLEIN	PANCREATIC LIPASE	0	0	0	0
	INTESTINAL LIPASE	0	0	0	0

Table IV: Comparison of positional specificity of intestinal and pancreatic lipases
The incubation media (25°C; 0.5 ml total volume) contained (final concentrations) : potassium phosphate buffer 50 mM pH 6.0, sodium taurocholate 1 mM, monoolein 25 mM, and 400 Units of homogeneous pig pancreatic lipase or 31 Units of 150 fold purified intestinal lipase (as measured with tributyrin as substrate). At the times indicated, 0.1 ml aliquots were removed and unhydrolyzed monoolein was extracted according to Bligh and Dyer (17). Glycerol was assayed in the aqueous phase after periodate oxidation and titration of the liberated formaldehyde by chromotropic acid (18). Control experiments were performed in the absence of enzyme; under these conditions no chemical isomerization of 2-monoolein occured (18) and no glycerol was released, but the monoolein still remaining in the aqueous phase (4% at all times) was substracted from the reported values. The results (from duplicate experiments) are expressed in percentage of hydrolysis of the substrate initially present.

---

of ingested fat. The presence of a gastric lipase in adult man, with an acidic optimum pH, has been described and characterized by Cohen et al. (6); this lipase is active against short- and medium-chain triglycerides. It is proposed that, besides gastric lipase, the intestinal lipase could also contribute to the hydrolysis of these neutral lipids. In man the quantitative

contribution of gastric and intestinal lipases is difficult to evaluate as compare to pancreatic lipase (9). In rat recent experiments indicate that the intestinal lipase may represent up to 20% of the total lipase activity present in the intestinal contents. Thus, if the situation for rat could be extrapolated for man, the contribution of the intestinal lipase to the absorption of neutral lipids could become significant, particularly in pathological situations.

ACKNOWLEDGMENTS The authors wish to thank Dr. M. Balerna for help concerning polyacrylamide gel electrophoresis. This work was supported by grants from the "Délégation Générale à la Recherche Scientifique" (Convention n° 73-7-1205), from the "Fondation pour la Recherche Médicale Française) and from the "Centre National pour la Recherche Scientifique" (Laboratoire Associé 201).

REFERENCES

1- Desnuelle,P. (1972) in The Enzymes (Boyer,P.ED), vol. 7 575-616, Academic Press, New York and London.
2- Di Nella,R.R., Meng,H.C. and Park,C.R. (1960), J. Biol. Chem. 235, 3076-3081.
3- Senior,J.R. and Isselbacher,K.J. (1963), J. Clin. Invest. 42, 187-195.
4- Pope,J.L., Mc Pherson,J.C. and Tidwell,H.C. (1966), J. Biol. Chem. 241, 2306-2310.
5- Hamosh,M. and Scow,R.O. (1973), J. Clin. Invest. 52, 88-95.
6- Cohen,M., Reginald,G.H., Morgan,M.B. and Hofman,A.F. (1971), Gastroenterology 60, 1-15.
7- Fernandez,J., Van de Kamer,J.H. and Weijers,H.A. (1962), J. Clin. Invest. 41, 488-495.
8- Sheldon,W. (1964), Arch. Dis. Child. 39, 268-271.
9- Figarella,C., Négri,G.A. and Sarles,H. (1972), Biochim. Biophys. Acta 280, 205-210.
10- Sémériva,M., Benzonana,G. and Desnuelle,P. (1967), Biochim. Biophys. Acta 144, 703-709.
11- Sémériva,M., Dufour,C. and Desnuelle,P. (1971) Biochemistry 10, 2143-2149.
12- Lowry,O.H., Rosebrough,N.J., Farr,A.L. and Randall,R.J. (1951), J. Biol. Chem. 193, 265-275.
13- Maurer,H.R. (1971) in Disc Electrophoresis and related techniques of polyacrylamide gel electrophoresis, p. 44, W. de Gruyter, Berlin and New York.
14- Ailhaud,G.P., Vagelos,P.R. and Goldfine,H. (1967) J. Biol. Chem. 242, 4459-4465.
15- Verger,R., DeHaas,G.H., Sarda,L. and Desnuelle,P. (1969) Biochim. Biophys. Acta 188, 272-282.
16- Maylié,M.F., Charles,M. and Desnuelle,P. (1972) Biochim. Biophys. Acta 276, 162-175.
17- Bligh,E. and Dyer,W. (1959) Canad. J. Biochem. 37, 911
18- Ailhaud,G., Samuel,D., Lazdunski,M. and Desnuelle,P. (1964) Biochim. Biophys. Acta 84, 643-664.