LONG-CHAIN TRIGLYCERIDE LIPASES OF PIG LIVER

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1. Introduction

It appears that the recently established capacity of rat liver homogenates to hydrolyze long-chain triglycerides [1,2] is due primarily to the presence of a lysosomal lipase with an acidic pH optimum [3–5] and a microsomal lipase exhibiting maximal activity at alkaline pH values [6,7]. There is less conclusive evidence for the presence of another distinct lipase in the plasma membrane [4,8,9] as well as an inactive lipoprotein lipase [10]. However, it has also been proposed [11] that liver lipase is a complex of liver esterase and lipid rather than a separate enzyme. Some of these conflicting results indicate clearly the necessity for further studies on the characterization and differentiation of liver lipolytic enzymes which obviously play a very significant role not only in intracellular catabolism but also in the uptake of triglycerides by the liver.

This communication describes the pH optima, time courses of hydrolysis and the effects of various compounds on the pig liver lipolytic activities and presents the evidence for their subcellular localization.

2. Experimental procedure

2.1. *Materials*

Commercially available olive oil was purified by column chromatography on silicic acid [12]; thin layer chromatography of purified olive oil preparation on silica gel G revealed a single spot with an R_f value corresponding to triglyceride. The preparation was stored under nitrogen at 0°. Substitution of triolein (Grade A, Calbiochem, Los Angeles, Calif.) for the purified triglyceride preparation from olive oil had no significant effect on the results.

2.2. *Enzyme assay*

To prepare the substrate emulsion, 1.2 mmoles of purified triglyceride preparation were mixed in a 20 ml glass beaker with 12 mM sodium taurocholate solution to a final volume of 10 ml, and the mixture was submitted to three successive cycles of vigorous stirring with a micro stirring bar for 3 min and sonication for 20 sec (Bronwill Biosonik II, cathenoidal tip, 50% power output, Bronwill Scientific, Rochester, New York). The emulsion was stable for several hours.

The incubation mixture consisted of 0.5 ml 0.1 M glycyl-glycine buffer of appropriate pH value, 0.5 ml triglyceride emulsion containing 60 μmoles substrate (sufficient to ensure maximal activity) and 0.5 ml enzyme preparation.

Incubations were carried out under nitrogen in culture tubes with teflon-lined caps for 15–60 min at 37° in a shaking water bath. Zero-time controls and samples containing the incubation mixture without triglyceride were included in each set of experiments. Fatty acids were extracted by the method of Dole [13] and titrated with 0.01 N NaOH in a single-phase system to pH 9.8 using Radiometer automatic titration equipment [14]. Palmitic acid (Mann Research Laboratories, N.Y., 99% pure by TLC) dissolved in heptane was used as standard for titration

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of fatty acids. Specific activities were expressed as μmoles of fatty acids released per mg of protein per hour.

2.3. *Subcellular fractionation*

Fresh pig liver was homogenized in a ten-fold volume of 0.25 M sucrose containing 1 mM EDTA, pH 7.2, with a Sorvall "Omni-Mixer" homogenizer (J. Sorvall, Inc., Norwalk, Conn.) for 60 sec at 50% energy output and 20 sec at full speed. The homogenate was filtered through 4-ply cheese cloth and the filtrate was subjected to differential centrifugation at the following speeds: 1020 *g* for 10 min; 3300 *g* for 10 min; 14,600 *g* for 30 min; 130,000 *g* for 30 min, with each successive sediment being washed one time under the same conditions. This procedure resulted in the isolation of nuclear (N), mitochondrial (M), light-mitochondrial (L), microsomal (P) and soluble (S) fractions, respectively [15]. Centrifugations were carried out at 4°.

Lysosomes were isolated from the light mitochondrial fraction according to the procedure by Ragab et al. [16]. The pellet from the second density gradient centrifugation ("lysosomal pellet 2") was tested by determining the activities of several marker enzymes such as arylsulfatase, cathepsin D and glucose-6-phosphatase and used for assaying the lysosomal liplytic activity.

All subcellular preparations were stored at −18° and thawed immediately before each incubation; repeated freezing and thawing of fractions was avoided.

3. Results and discussion

Experiments with pig liver homogenate as the enzyme source showed two different pH optima for lipolytic activity: a relatively sharp maximum at pH 4–4.8 and a broader peak at pH 7–8.3. The release of fatty acids after incubation for 60 min at the alkaline pH maximum was approximately one-half the amount liberated at the acid pH optimum. In agreement with similar reports for rat liver [1,4,5], this observation suggests that pig liver contains at least two distinct lipolytic activities involved in the breakdown of long-chain triglycerides.

3.1. *Subcellular distribution of lipases*

The highest specific activity of acid lipase was found in the light mitochondrial fraction. The still

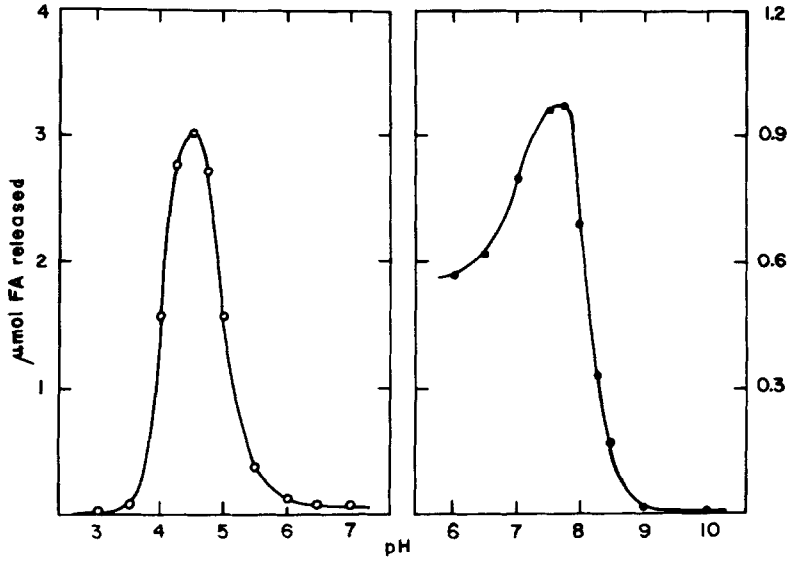

Fig. 1. Effect of pH on lipolytic activity of lysosomes and microsomes. Buffer: 0.1 M glycyl-glycine. Lysosomal protein (0.47 mg) was incubated for 60 min in the presence of 2 mg Triton X-100/test (o—o); microsomal protein (1.08 mg) was incubated for 15 min without any additives (•—•); the assay was done as described under Experimental procedure.

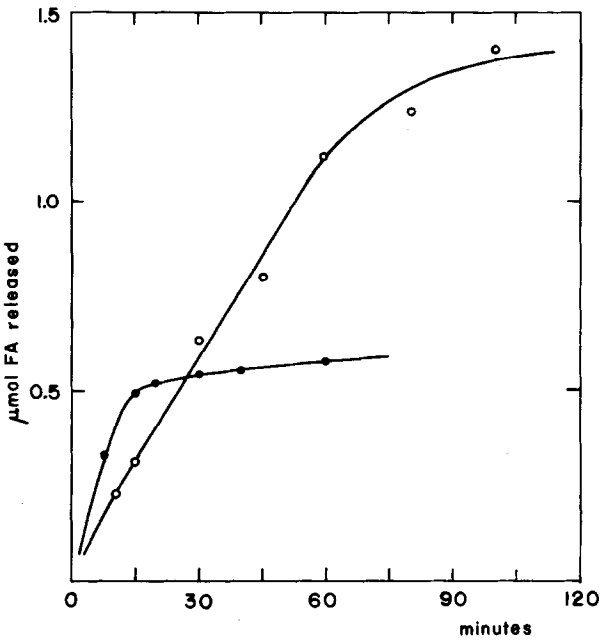

Fig. 2. Time course of lipolysis by lysosomes and microsomes. Lysosomal protein (0.51 mg) was incubated at pH 4.25 (open circles); microsomal protein (1.08 mg) was incubated at pH 8.0 (closed circles); the assay was performed as described under Experimental procedure.

appreciable activities of mitochondrial and microsomal fractions were higher than that of the soluble fraction. This distribution pattern parallels very closely that of acid phosphatase, which is considered to be a typical lysosomal enzyme [3,15].

The isolated and purified lysosomal fraction was characterized by an 83-fold increase in the activity of arylsulfatase and a 12-fold increase in the activity of cathepsin D. The lipolytic activity of this preparation was increased 18-fold (3 µmoles fatty acids/mg protein/60 min) compared to the crude homogenate; it exhibited a sharp pH optimum at 4.5 (fig. 1). These results indicated clearly the lysosomal origin of acid lipase.

Mahadevan and Tappel [3] demonstrated the presence of an acid lipase in the lysosomes of rat liver and Guder et al. [4] achieved a 300-fold purification of this enzyme by centrifuging sonicated Tritonfilled lysosomes. The pH optima of these preparations were 4.2 and 4.5–5, respectively.

Table 1
Effect of additives on lipolytic activities of lysosomes and microsomes

Addition compound	Final concentration	% of control activity	
		Lysosomes	Microsomes
EDTA	1×10^{-3} M	127	113
Triton X-100	2 mg/test	203	4
Triton X-100	8 mg/test	23	0
Ca^{2+}	5×10^{-3} M	99	18
NH_4^+	5×10^{-2} M	109	19
Iodoacetamide	5×10^{-2} M	96	91

The lysosomal preparations were assayed fro 60 min at pH 4.5 and the microsomal preparations for 15 min at pH 7.5.

The highest specific activity of alkaline lipase was found in the microsomal fraction, which was characterized by an 8-fold increase in the activity of glucose-6-phosphatase used as the marker enzyme for this subcellular fraction. The pH optimum of microsomal lipase was 7.5–7.8; however, the activity rapidly decreased at pH values greater than 8 and was essentially zero at pH 9 (fig. 1). Carter [6] studied a similar lipolytic activity at pH 7.4 (the pH optimum was not determined) in rat liver. Mahadevan and Tappel [3] and Guder et al. [4] found that the pH optima for this lipolytic activity were 8.6 and 8.5, respectively.

3.2. *Effect of time and enzyme concentration*

There was a striking difference in the time dependence between the acid and alkaline lipolytic activities (fig. 2). Lipolysis by lysosomal lipase was linear for more than 60 min; on the other hand, microsomal lipase released fatty acids at a linear rate for only 10–15 min. Similar results were reported for the lysosomal [3] and the microsomal [6] lipase in rat liver.

Release of fatty acids was directly proportional to the amount of protein present up to at least 0.65 mg lysosomal protein/test (incubation time: 60 min) or at least 1.65 mg microsomal protein/test (incubation time: 15 min).

3.3. *Effects of additives*

The effect of various compounds on the acid and alkaline lipolytic activities is shown in table 1. Calcium

and ammonium ions were found to be the most effective additives for differentiating between the lysosomal and microsomal lipases: they inhibited the microsomal lipase, but had no effect on the lysosomal lipase. At low concentration, Triton X-100 activitated the lysosomal and inhibited the microsomal lipase. However, at higher concentration Triton inhibited both lipases. EDTA activated both lipolytic activities slightly. Iodoacetamide had no effect on either lipolytic activity.

4. Conclusion

Results of this study indicate that pig liver contains at least two long-chain triglyceride lipases. One of these exhibits an acidic pH optimum and is localized in lysosomes; the other displays an alkaline pH-optimum and is present in microsomes.

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References

[1] H. Vavřinkova and B. Mosinger, Physiol. Bohemoslov. 14 (1965) 46.
[2] A.C. Olson and P. Alaupovic, Biochim. Biophys. Acta 125 (1966) 185.
[3] S. Mahadevan and A.L. Tappel, J. Biol. Chem. 243 (1968) 2849.
[4] W. Guder, L. Weiss and O. Wieland, Biochim. Biophys. Acta 187 (1969) 173.
[5] K. Hayase and A.L. Tappel, J. Biol. Chem. 245 (1970) 169.
[6] J.R. Carter, Biochim. Biophys. Acta 137 (1967) 147.
[7] Y. Biale, E. Gorin and E. Shafrir, Biochim. Biophys. Acta 152 (1968) 28.
[8] J.A. Higgins and C. Green, Biochem. J. 99 (1966) 631.
[9] J.A. Higgins and C. Green, Biochim. Biophys. Acta 144 (1967) 211.
[10] P.A. Mayes and J.M. Felts, Biochem. J. 108 (1968) 483.
[11] H. Okuda and S. Fujii, J. Biochem. 64 (1968) 377.
[12] M.G. Horning, E.A. Williams and E.C. Horning, J. Lipid Res. 1 (1960) 482.
[13] V.P. Dole, J. Clin. Invest. 35 (1956) 150.
[14] J.D. Schnatz, J. Lipid Res. 5 (1964) 483.
[15] C. deDuve, B.C. Pressman, R. Gianetto, R. Wattiaux and F. Appelmans, Biochem. J. 60 (1955) 604.
[16] H. Ragab, C. Beck, C. Dillard and A.L. Tappel, Biochim. Biophys. Acta 148 (1967) 501.