TRIGLYCERIDE LIPASE ACTIVITY IN SUBCELLULAR FRACTIONS FROM BEEF HEART

Inger SCHOUSBOE, P.D. BARTELS and P.K. JENSEN
Department of Biochemistry C, University of Copenhagen, 71, Rådmandsgade, DK-2200 Copenhagen, Denmark

Received 2 July 1973

1. Introduction

Lipolytic activity in the heart has so far been studied mainly with respect to lipoprotein lipase and the role of this enzyme in transfer of fatty acids from plasma triglycerides to the muscle cells. Interest in transfer of fatty acids from intracellular stores of triglyceride to mitochondria was stimulated in the present laboratory, when membrane bound lipid particles were isolated from beef heart homogenates [1]. The triglyceride content of these particles was 90–95%, 3–5% was protein and the electron microscopic appearance of the isolated particles was similar to that of particles *in situ* in beef heart muscle cells. Here as well as in other heart muscle preparations they are seen surrounded by mitochondria [2, 3], which they probably furnish with fatty acids in connection with triglyceride hydrolysis [cf. 4]. On this background it was decided to study the intracellular localization of triglyceride lipase (TGL) activity in beef heart muscle cells, presently the only material from which lipid particles of this kind have been isolated.

This letter reports the distribution of neutral to alkaline TGL activity among subcellular fractions prepared from beef heart cytoplasmic extracts (i.e. homogenates freed of nuclei and cell debris [5]). One half of the recovered TGL activity was in the soluble fraction, the other half was evenly distributed between the microsomal and mitochondrial fractions. However, contamination of the mitochondrial fraction with microsomal protein most likely accounts for all TGL activity in the mitochondrial fraction. As judged by the effect of serum, NaCl and heparin, lipoprotein lipase is present in all fractions, but the results indicate that other lipases contribute to the overall TGL activity.

Lipid particle fractions were also prepared and used as substrate for TGL activity in the other subcellular fractions. Release of fatty acids could be demonstrated with the microsomal fraction. The lipid fraction itself had some TGL activity (with added triglyceride as substrate) which did not appear to be caused by microsomal contamination.

2. Material and methods

2.1. *Subcellular fractions*

Homogenates prepared according to Crane et al. [6] were centrifuged at 1600 *g* for 15 min. The supernatant was filtered through double layered cheese cloth (cytoplasmic extract) and used for preparation of a mitochondrial, a microsomal and a soluble fraction by conventional techniques (15 000 *g* for 15 min for the mitochondrial fraction, and 100 000 *g* for the microsomal and soluble fraction). Lipid particle fractions were prepared as previously described [1] by continous centrifugation of large volumes of diluted cytoplasmic extracts.

2.2. *TGL assay*

The amount of fatty acids hydrolyzed from triglyceride was measured radiochemically, either by including glyceryl tri(oleate-1-^{14}C) in the substrate suspension [7] or by complex formation between ^{63}Ni and fatty acids separated from the incubation mixture [8].

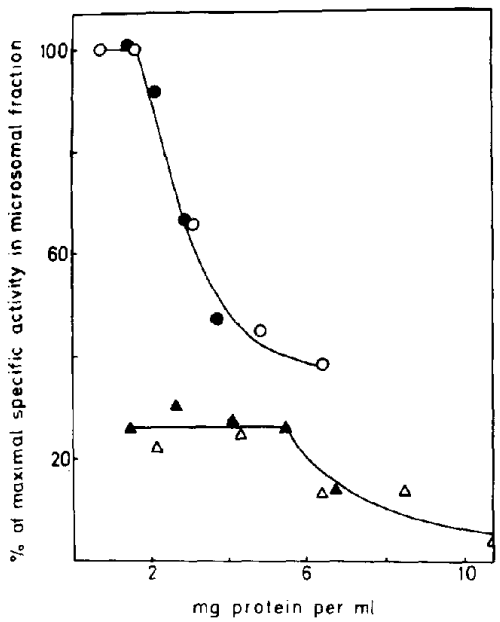

Fig. 1. Influence of microsomal (● ○) and mitochondrial (▲ △) protein concentration on specific TGL-activity. Triglyceride concentration 4.5 (● ▲) and 8.5 (○ △) mM.

Substrate suspensions were prepared fresh from 3.0 ml bovine serum, 1.0 ml 1% (w/v) bovine serum albumin and 200 μl purified [7] triglyceride (5 μCi [^{14}C]triolein in olive oil (77% triolein)). This mixture was sonificated for 4 min at 0–5°C with the microtip of a Branson sonifier at 75 W.

Incubation mixtures included 60 μmol Tris–HCl, 5–10 μmol triglyceride either suspended with protein or as lipid particle triglyceride, and 0.2–2 mg protein from subcellular fractions in a total volume of 500 μl. The reaction was stopped by addition of 100 μl M phosphoric acid and rapid cooling.

Extraction and separation of fatty acids from incubation mixtures were performed according to Kaplan [7]. Labeling of fatty acids from incubations without labeled triolein was made as described by Ho [8]. Radioactivity measurements in dioxane based scintillation fluids [7] were performed with Nuclear Chicago Mark II equipment.

Rates of fatty acid release were constant during incubations and linear with the concentration of added triglyceride up to 15 mM with the 'classical' subcellular fractions as enzyme source, whereas saturation was obtained beyond 5 mM (added) triglyceride with the lipid particle fraction. Linearity with protein concentration was limited to values below 6 mg/ml incubation mixture for the mitochondrial and to 2 mg/ml for the microsomal fraction (fig. 1).

2.3. *Other methods*

Cytochrome oxidase was assayed as described by DeDuve et al. [5], and Rotenone-insensitive NADPH-cytochrome *c* reductase as described by Sottocasa et al. [9], but with a lower (0.15 mM) Rotenone concentration [10]. Protein was determined *ad modum* Lowry et al. [11].

2.4. *Chemicals*

Cytochrome *c* and NADPH were from Boehringer (Germany), bovine serum albumin and heparin from Sigma (USA), Pantozym from Wander (Switzerland) and glyceryl tri(oleate-1-^{14}C) and $^{63}NiCl_2$ from The Radiochemical Centre (Amersham, England). Bovine serum was kindly supplied by Professor P. Halskov Sørensen, The Royal Veterinary and Agricultural University, Copenhagen.

3. Results and discussion

3.1. *Distribution of TGL activity among subcellular fractions*

TGL activity in the extracts prepared throughout the investigation varied from 0.5–3 μmol fatty acids released per hr per g tissue. This is about 10 times less than the activity found in comparable preparations from rat heart [12]. The authors are not aware of published figures for beef heart preparations.

Table 1 lists the distribution of the activity among subcellular fractions obtained from 5 cytoplasmic extracts. About 90% of the activity was recovered with 50% in the soluble and the rest evenly distributed between the two particulate fractions. The same relative distribution was found in the above mentioned rat heart preparations. The activity of the soluble fraction includes activity found to be present in the lipid particle fraction, which had to be prepared separately, because of the low yield and special techniques involved. For these reasons figures for this fraction have not been included in table 1. It is unlikely that more than 5% of the total TGL-activity

Table 1
Distribution of protein and TGL-activity among subcellular fractions.

Fraction	Protein*	TGL-activity*	Relative specific activity**
Mitochondrial	11 ± 1.0	22 ± 3.0	2.1 ± 0.4
Microsomal	3.5 ± 0.15	22 ± 2.0	6.5 ± 0.6
Soluble	84 ± 1.5	44 ± 4.0	0.5 ± 0.15

* Percent of total in cytoplasmic extract. Average from 5 preparations with standard error of the mean.
** Specific activity of TGL-activity in cytoplasmic extract set to 1.0.

of the cytoplasmic extract adhere to this fraction. The specific activity was about the same as in the mitochondrial fraction (cf. fig. 2).

As prepared the fractions were crude and cross contamination had to be evaluated. Approximately 30% of the protein in the microsomal fraction could be identified as mitochondrial by the cytochrome oxidase activity of the microsomal fraction. With the highest specific activity found in the microsomal fraction this contamination would not change the relative distribution of TGL activity markedly. Microsomal contamination of the mitochondrial fraction, however, presented a more serious problem, since 30–35% contamination would suffice to account completely for the activity in the mitochondrial fraction and would also fit the observation that the specific activity of this fraction was constant over a 3 times wider range

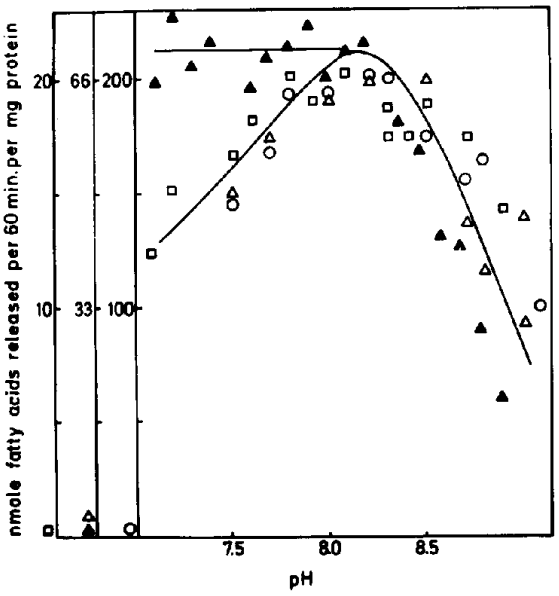

Fig. 2. Influence of pH on TGL-activity in soluble (□) mitochondrial (△) lipid particle (▲) and microsomal (□) fractions. Buffers: pH 7–7.4 Tris–malate, 7,4–9 Tris–HCl. Triglyceride concentration: 5 mM. (Note the different ordinate values).

than the 3 times higher specific activity of the microsomal fraction.

Unfortunately the activity of the microsomal marker enzyme: Rotenone intensive NADPH-cytochrome *c* reductase [10] was found to be so low in beef heart microsomal fractions (10 times lower than in rat heart microsomal fraction assayed in parallel

Table 2
Influence of serum and NaCl on TGL-activity in subcellular fractions.

Incubation medium	Relative TGL-activity*			
	Mitochondrial fraction	Microsomal fraction	Soluble fraction	Lipid particle fraction**
Serum omitted	33 ± 3.0 [3]	37 ± 4.0 [4]	35	–
Serum albumin omitted, replaced by gum arabic	52 ± 1.0 [3]	58 ± 4.0 [4]	47	–
NaCl added to 1 M	27 ± 8.0 [3]	27 ± 4.0 [4]	19 ± 3 (6)	25 ± 1.0 [3]

* Percent of activity with complete medium. Average with standard error of the mean, number of experiments in brackets.
** With added triglyceride.

Table 3
Release of fatty acids during incubation of lipid particle fraction with various lipase preparations.

Source of lipase	mg protein/ml	mg Lipid particle triglyceride/ml	nmole Fatty acid released per ml at		
			0 min	60 min	Difference
No lipase added	(0.1)	2	74 ± 11*	68 ± 14*	0
No lipase added	(0.25)	5	270	270	0
Hog pancreas (Pantozym)	2.0	2	20	1180	1160
Bovine milk**	2.0	2	50	390	340
Microsomal fraction	2.0	none	100	120	20
Microsomal fraction	2.0	2	140	440	300
Microsomal fraction	1.0	5	210	400	190

* Average of 5 experiments with standard error of the mean.
** Supernatant after centrifuation at 80 000 *g* for 2 hr of raw skimmilk made 0.75 M with respect to NaCl [16].

experiments) that only a very rough estimate of microsomal contamination of the mitochondrial fraction in order of 20–50% could be obtained.

Since two consecutive washings of the crude mitochondrial fraction left only 7% of the TGL activity (less than 2% of the activity in the cytoplasmic extract) in a fraction of 'heavy' mitochondria sedimented at 10 000 *g* for 15 min, and since no differences could be observed in the influence of pH and NaCl on the activity in the mitochondrial and microsomal fractions (cf. fig. 2 and table 2), it has been concluded that the activity in the mitochondrial fraction is caused by microsomal contamination.

Contamination with microsomal protein might also have explained the TGL activity found in the lipid particle fraction. Washing procedures [1], which are more effective in removing microsomal protein from lipid particles than from mitochondria, did reduce the total activity, but the specific activity doubled, so that more than 50% of the protein would have to be microsomal in order to account for the activity. Since the activity also showed a pH dependence different from that of the microsomal (fig. 2), these results were taken to indicate that some TGL activity is located in the lipid particle membrane.

3.2. *Nature of TGL activity in subcellular fractions*

TGL activity was routinely assayed in the presence of bovine serum and at pH 8.2 in order to include contribution from lipoprotein lipase. This enzyme has previously been demonstrated in extracts of beef hearts [13, 14], but without quantitation of total activity. It has been suggested that it might be involved also in utilization of endogenous triglyceride stores as well as in provision of fatty acids from plasma triglycerides [15].

Omission of serum or its replacement with gum arabic as well as addition of NaCl to the complete medium led to reduction of TGL activity in all fractions (table 2) Heparin stimulated activity both in the soluble and microsomal fraction 20–40%. It seems certain therefore, that all fractions contain lipoprotein lipase. However, the presence of serum was not an absolute requirement for activity, nor was the inhibition by NaCl complete or nearly complete as has been observed with other lipoprotein lipase preparations. The stimulation by heparin was also much lower than the doubling of activity observed e.g. with the soluble fraction from rat heart preparations [12]. These observations indicate that other lipases also contribute to the overall TGL activity, but more direct evidence has so far been found only in the pH dependence of the lipid particle fraction, which indicated the presence of neutral lipase (fig. 2).

3.3. *Triglyceride of the lipid particle fraction as substrate for TGL activity*

In order to see to what extent the triglyceride in the lipid particle could be utilized as substrate for lipases, experiments were performed in which the artificial triglyceride suspension was replaced by the lipid particle fraction (table 3). Hydrolysis of less than 0.5% of the fractions' triglyceride was to be expected in 1 hr incubations as a result of the fractions own TGL activity assayed with added triglyceride. This was confirmed by the absence of any measurable fatty acid

release in incubations of the lipid particle fraction alone. Pancreatic and milk lipases were active with the lipid particle fraction as substrate and the particle membrane thus presents no barrier to these enzymes. The amounts of fatty acids released in incubations with the microsomal fraction were of the same order of magnitude as in incubations with labeled triolein suspended with serum proteins (cf. fig. 2). Apparently the triglyceride in the membrane bound lipid particle is neither more nor less readily available for microsomal lipase than triglyceride suspended with serum proteins. Similar results (not presented) were found with respect to the soluble lipase.

Nevertheless it seems worthwhile to make use of the lipid particle fraction as substrate in attempts to further characterize the lipases in the heart. It is unlikely that the particle membrane might play a role in regulation – hormonal or otherwise – of their activity.

Acknowledgement

This work was supported by grants no. 511-569, 511-1075 and 512-1052 from the Danish State Science and Medical Research Councils.

References

[1] Christiansen, K. and Jensen, P.K. (1972) Biochim. Biophys. Acta 260, 449.
[2] Fawcett, D.B. (1966) in: The Cell. An Atlas of fine Structure (Saunders, W.B., ed.) pp. 102–105, 307–318, Philadelphia.
[3] Stein, O. and Stein, Y. (1968) J. Cell Biol. 36, 63.
[4] Olson, R.E. and Hoeschen, R.J. (1967) Biochem. J. 103, 796.
[5] DeDuve, C., Pressmann, B.C., Gianette, R., Wattiaux, R. and Appelmans, F. (1955) Biochem. J. 60, 604.
[6] Crane, F.L., Glenn, J.J. and Green, D.E. (1956) Biochim. Biophys. Acta 22, 475.
[7] Kaplan, A. (1970) Anal. Biochem. 33, 218.
[8] Ho, R.J. (1970) Anal. Biochem. 36, 105.
[9] Sottocasa, G.L., Kuylenstierna, B., Ernster, L. and Bergstrand, A. (1967) J. Cell Biol. 32, 415.
[10] DeJong, J.W. and Hülsmann, W.C. (1970) Biochim. Biophys. Acta 197, 127.
[11] Lowry, O.H., Rosebough, N.J., Farr, N.J. and Randall, R.J. (1951) J. Biol. Chem. 193, 265.
[12] Gartner, S.L. and Vahouny, G.V. (1966) Am. J. Physiol. 211, 1063.
[13] Korn, E.D. (1955) J. Biol. Chem. 215, 1.
[14] Iselin, B. and Schuler, W. (1957) Helv. Physiol. Acta 15, 14.
[15] Kreisberg, R.A. (1966) Am. J. Physiol. 210, 385.
[16] Downey, W.K. and Andrews, P. (1969) Biochim. J. 112, 559.