A SIMPLE PURIFICATION PROCEDURE FOR RAT HEPATIC LIPASE

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1. Introduction

Lipoprotein lipase (LPL, EC 3.1.1.3.) of the extrahepatic tissues is responsible for the hydrolysis of triacylglycerols transported in plasma chylomicrons and very low density lipoproteins [1,2]. This exo-enzyme can be released into the circulation by intravenous injection of heparin or similar polyanions [3]. In addition to the lipoprotein lipase, post-heparin plasma contains a triacylglycerol lipase of hepatic origin [4]. The hepatic lipase also hydrolyzes monoacylglycerols and phospholipids [5,6]. The role of the hepatic lipase in the lipoprotein metabolism is not known. It has been suggested that the enzyme could participate in the hydrolysis of the lipids in 'remnant' lipoproteins [7]. The activity of the hepatic lipase is not lowered in hypertriglyceridaemia, and it does not correlate to the plasma level of triacylglycerol [1]. Accordingly, the hepatic lipase cannot be the rate-limiting factor in plasma triacylglycerol removal. It has also been proposed that the hepatic lipase could mediate the breakdown of lipoprotein monoacylglycerols [8,9].

As the role of the hepatic lipase in lipoprotein metabolism is far from resolved, we felt it necessary to purify the enzyme in sufficient quantities to enable unambigious in vitro studies. We now describe a simple purification procedure for the rat hepatic lipase using heparin-containing liver perfusates as a starting material.

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2. Materials and methods

2.1. *Materials*

Tri[1-^{14}C]oleoylglycerol (59 μCi/μmol) was obtained from the Radiochemical Centre, Amersham, Bucks and the unlabelled triolein from the Sigma Chemical Co., St Louis, Mo. Monooleyl [^{3}H]glycerol was a gift from Drs Per Belfrage and Tornqvist, Lund and the corresponding unlabelled lipid was obtained from NU Check. Prep. Inc., USA. Sepharose 4B was from Pharmacia Fine Chemicals, Uppsala and Ultrogel AcA 34 from LKB-Products, Bromma. Heparin was from Medica, Helsinki. Bovine serum albumin was supplied by Armour Pharm. Co., Eastbourne, Sussex.

2.2. *Assay for lipase activity*

The determination of triacylglycerol lipase activity was carried out with the sonified emulsion of triolein and gum arabic at 1.0 M NaCl as in [13].

Monoacylglycerol hydrolase activity was determined as in [14] using Triton X-100 as emulsifier.

2.3. *Animals*

Male Sprague-Dawley rats (300–350 g) were used in the experiments. They had free access to tap water and commercial laboratory chow (Hankkija Ltd, Finland) prior to the experiments.

2.4. *Liver perfusion*

Rats were anaesthesized by intraperitoneal injection of Nembutal® (25 mg/kg). Their portal vein was cannulated and the liver was flushed free from blood with 250 ml Krebs-Ringer bicarbonate buffer

(pH 7.4) saturated with 95% O_2–5% CO_2 (KRB). The chest was opened and the inferior caval vein was cannulated just above the liver. The vein was then ligated above the right kidney, and the liver was connected to a recirculating perfusion apparatus [10]. To release the hepatic lipase into the perfusate, the medium was changed to 50–100 ml KRB containing 40 IU heparin/ml, and was allowed to circulate for 5 min. The same medium was used for consecutive perfusions of 6 rat livers.

2.5. *Enzyme purification*

The heparin containing perfusate from 6 rat livers was applied to a column (1.2 × 6 cm) of Sepharose 4B containing covalently-bound heparin [11] equilibrated with 0.15 M NaCl in 5 mM sodium barbital buffer (pH 7.4) containing 20% (v/v) glycerol. After enzyme application the column was first washed with 60 ml 0.15 M NaCl in 5 mM sodium barbital buffer (pH 7.4) containing 20% (v/v) glycerol and 0.1% (v/v) Triton X-100, followed by a wash with 60 ml same buffer but without Triton X-100 [12]. The enzyme was eluted with a linear NaCl gradient from 0.4–2.0 M in 5 mM sodium barbital buffer (pH 7.4) containing 20% (v/v) glycerol, total vol. 100 ml. The fractions (6 ml) were assayed for triacylglycerol lipase and monoacylglycerol hydrolase activity, and those containing the enzyme activity were combined.

The lipase present in the pooled fractions was precipitated by adding solid ammonium sulphate to 100% saturation at +4°C. After standing overnight at +4°C the precipitate was collected by centrifugation for 30 min at 37 000 × *g*. The precipitate was dissolved in 2 ml 5 mM sodium barbital buffer (pH 7.4) containing 0.1 M methyl-α-D-glucopyranoside, 0.1 M D-galactose, 2.0 M NaCl and 0.1% (v/v) Triton X-100. After dialysis against the same buffer for 1 h at +4°C it was subjected to gel filtration in a column (1.6 × 84 cm) of Ultrogel AcA 34. The column was eluted at 5 ml/h and 1.2 ml fractions were collected.

2.6. *Other methods*

Protein was measured by the Lowry method [15] or by a modified method for determination of μg quantities [16]. Polyacrylamide slab-gel electrophoresis was performed by the Laemmli method [17].

3. Results

3.1. *Perfusion of rat livers*

The total release of triacylglycerol lipase activity into the heparin-containing perfusate from 6 rats was 2483 ± 451 μmol FFA.h^{-1} (mean ± SEM, 2 perfusates), and that of monoacylglycerol hydrolase activity 888 ± 163 units. This represents a mean release of 34.5 ± 6.5 μmol FFA.h^{-1} of triacylglycerol hydrolase activity per gram of rat liver assuming that the mean liver weight was 12 g. The protein concentration of the perfusates was 194.1 ± 24.6 μg/ml corresponding to spec. act. 102.4 ± 23.8 μmol FFA.mg protein^{-1}. The release of the hepatic lipase by heparin into KRB resulted in starting material with 74-fold higher specific activity than that observed in the post-heparin plasma. In post-heparin plasma the specific activity of the hepatic lipase was 1.39 ± 0.15 μmol FFA.mg protein^{-1} (mean ± SEM, $n = 5$).

3.2. *Enzyme purification*

Chromatography of the perfusate on heparin–Sepharose resulted in elution pattern illustrated in fig.1. Of the activity applied 9.6 ± 2.0% (mean ± SEM, 2 purifications) was not retained by the column and 3.5 ± 0.8% was recovered in the detergent-containing washes. Of the activity applied 28.8 ± 0.3% was eluted as a single peak at 0.8 M NaCl. Further triacylglycerol lipase activity, 15.6 ± 3.4% of that applied could be released from the column by 0.1% (v/v) Triton X-100 in 2.0 M NaCl. When the fractions were assayed under conditions optimal for lipoprotein lipase, 0.1 M NaCl and 10% (v/v) serum in the substrate, again only 1 triacylglycerol lipase peak was found corresponding to the activity measured in 1.0 M NaCl in the absence of serum. Monoacylglycerol hydrolase activity was eluted together with the triacylglycerol lipase activity. The triacylglycerol lipase : monoacylglycerol hydrolase activity ratio was 2.4 ± 0.2. The amount of protein in the peak fractions of the salt-eluted lipase was 7.7 ± 1.6 μg/ml corresponding to spec. act. 4070 ± 850 μmol FFA.h^{-1}.mg protein^{-1}. SDS–polyacrylamide gel electrophoresis showed 1 main band with app. mol. wt 62 500 (fig.2). Some faintly-staining components were, however, also seen.

For further purification the fractions with the

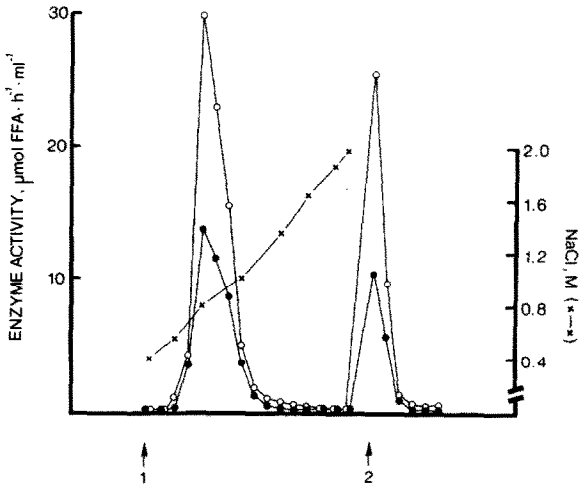

Fig.1. Chromatography of liver perfusate on heparin–Sepharose. The column was eluted with a linear gradient (total vol. 100 ml) of 0.4–2.0 M NaCl in 5 mM sodium barbital buffer (pH 7.4) containing 20% (v/v) glycerol, indicated with arrow 1. The fractions (6 ml) were analyzed for triacylglycerol lipase activity (○–○) with a substrate containing no serum at 1.0 M NaCl, and for monoacylglycerol hydrolase activity (●–●) as in section 2. After the gradient the column was eluted with 2.0 M NaCl in sodium barbital buffer (pH 7.4) containing 20% (v/v) glycerol and 0.1% (v/v) Triton X-100, indicated with arrow 2.

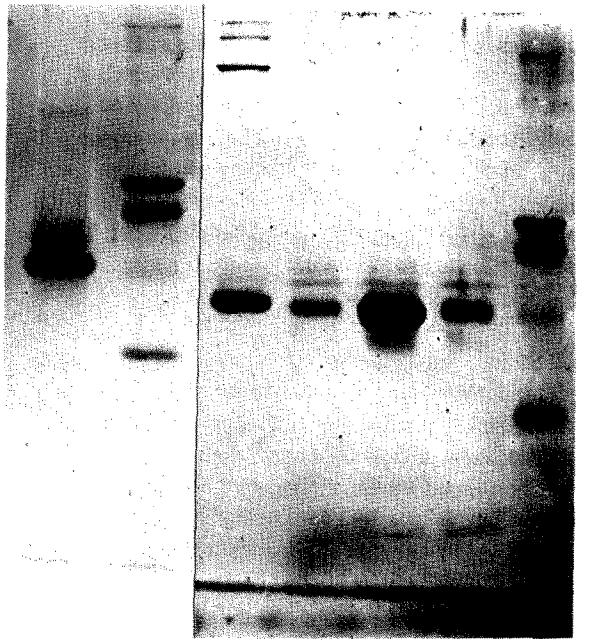

Fig.2. SDS–polyacrylamide gel electrophoresis of rat hepatic lipase at different stages of purification. Hepatic lipase (11 μg) eluted from heparin–Sepharose at 0.8 M NaCl (A), 12 μg dissolved ammonium sulphate precipitate (B), fractions 41 and 43 (7 μg) (C); 45 and 47 (19 μg) (D), and 49 and 51 (9 μg) (E), of the gel-filtrated lipase from fig.3. Standards were thyreoglobulin, transferrin, albumin, ovalbumin and hemoglobin.

highest specific activity of the salt-eluted lipase were combined and brought to 100% saturation at +4°C by adding solid ammonium sulphate. After standing overnight at +4°C the precipitate was collected and dissolved in a minimal volume of 5 mM sodium barbital buffer (pH 7.4) containing 2.0 M NaCl, 0.1 M D-galactose, 0.1 M methyl-D-glucopyranoside and 0.1% (v/v) Triton X-100. The recovery of the enzyme at the ammonium sulphate step was 110 ± 16%. The dissolved pellet was dialyzed against the same buffer for 2 h at +4°C, and subjected to gel filtration on an Ultrogel AcA 34 column. The enzyme eluted as a symmetrical peak with a recovery of 98 ± 4%, fig.3. The triacylglycerol lipase: monoacylglycerol hydrolase activity ratio was 2.5 ± 0.1. The molecular size derived from the gel filtration studies was 180 000 ± 5000. SDS-polyacrylamide gel electrophoresis revealed 1 major band with the same apparent molecular weight as after the affinity chromatography step, fig.2. The specific activity in the peak fraction was 5540 ± 170 μmol FFA.h^{-1}.mg protein^{-1}.

The results from 2 different purifications of rat liver triacylglycerol lipase are summarized in table 1. The liver perfusion with its initial washout of serum resulted in a 74-fold purification of hepatic lipase with respect to the post-heparin plasma. The heparin–Sepharose chromatography resulted in a 40-fold purification with a 29% recovery. At this step the specific activity of the enzyme was 4070 μmol FFA.h^{-1}.mg protein^{-1}. The gel filtration after the ammonium sulphate precipitation caused a 1.4-fold rise in the specific activity.

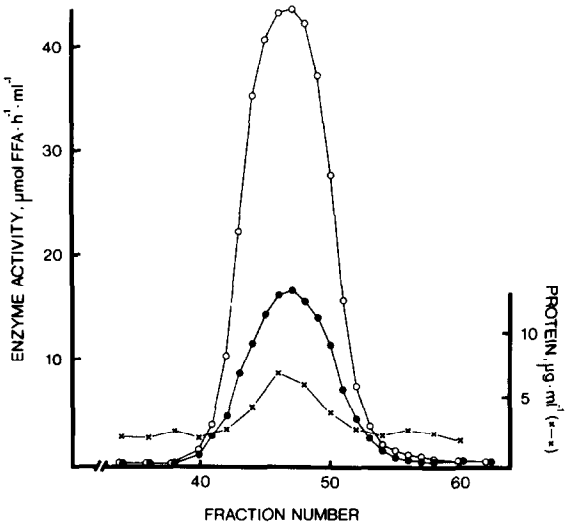

Fig.3. Gel filtration of rat hepatic lipase. The column of Ultrogel AcA34 was run at 5 ml/h and fractions (1.2 ml) were assayed for triglyceride lipase (o—o) and monoacylglycerol hydrolase (•—•) as in fig.1 legend.

4. Discussion

Chromatography on heparin–Sepharose of post-heparin plasma has been used for the purification of lipoprotein lipase and hepatic lipase [18]. Homogenous preparations have been, however, until recently difficult to obtain, since also another protein in post-heparin plasma, antithrombin III, has affinity to heparin–Sepharose [19,20]. This protein can be removed with the aid of low-affinity heparin [19]. By this procedure, only small quantities of purified lipases can be obtained from relatively large volumes of post-heparin plasma. Therefore, it is not suitable for the purification of rat post-heparin plasma lipases. Heparin-containing liver perfusates do not contain antithrombin III, as determined in double-diffusion experiments using anti-human antithrombin III serum, crossreacting with the corresponding rat protein. Accordingly, the low-affinity heparin–Sepharose chromatography step can be omitted when using liver perfusates as a starting material for the purification of rat hepatic lipase.

Lipoprotein lipase has been purified to homogeneity from bovine milk using a detergent containing heparin–Sepharose wash after enzyme application [12]. This method, however, cannot be applied to the purification of post-heparin plasma lipases, since after the detergent-wash lipoprotein lipase and hepatic lipase elute together with gradients of salt used to separate the 2 post-heparin plasma enzymes (T.K., P.K.J.K., unpublished results). Heparin perfusates of rat livers contain only hepatic lipase, as determined from the presence of only 1 lipase activity peak when the detergent wash is not used, as also by immunological methods (T.K., in preparation). By the use of detergent, hepatic lipase can be purified to

Table 1
Purification of the heparin-releasable triacylglycerol lipase from rat liver perfusates

	Specific activity μmol FFA.h^{-1}.mg^{-1}	Recovery (%)	Purification (-fold)	
Post-heparin plasma hepatic lipase	(1.39 ± 0.15)	–	–	(1)[a]
Heparin perfusates	102 ± 24	100	1	(74)
Chromatography on heparin–Sepharose	4067 ± 852	29	40	(2960)
Ammonium sulphate precipitation	–	32	–	(–)
Gel filtration on Ultrogel AcA 34	5539 ± 168	31	54	(4016)

[a] For comparison the calculated values for the rat postheparin plasma hepatic lipase are given in parentheses

Lipase activity was measured with a substrate containing no serum co-factor added at 1.0 M NaCl. Values are means ± SEM from 2 purifications

a high specific activity (4070 μmol FFA.h^{-1}.mg protein^{-1}) using only heparin–Sepharose chromatography of the perfusates. About 60 μg protein can be purified from liver perfusates of 6 rats. The specific activity is further increased to 5500 μmol FFA.h^{-1}.mg protein^{-1} by use of gel filtration, with ~100% recovery of the lipase activity. In our hands, the whole purification procedure generally takes 2–3 days.

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