PURIFICATION OF RAT ADIPOSE TISSUE LIPOPROTEIN LIPASE

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Received 29 June 1973

1. Introduction

Lipoprotein lipase (LPL), an enzyme present in adipose tissue, heart, mammary gland and other organs [1], is a triglyceride lipase (TGL) requiring an apolipoprotein from very-low-density lipoproteins, apoLp–glu, as cofactor for full activity [2,3]. When obtained from these tissues it has consistantly been shown that LPL is inhibited by 1 M sodium chloride. Triglyceride lipase activity from post-heparin plasma which is usually considered to be identical with LPL has been demonstrated to be only partially inhibited or even activated by NaCl. This variable effect of high ionic strength was attributed to the presence of more than one TGL in plasma [4]. Recently we have purified one TGL from human [5] and dog post-heparin plasma [6]. This enzyme differed from rat adipose tissue LPL by failing to show suppressed activity in 1 M NaCl and was similar to that of a TGL released by heparin from liver. In subsequent studies techniques were developed which allowed separation of two types of TGL from post-heparin plasma in one chromatographic step. Based on indirect evidence with inhibitors and activators it was assumed that the second of these plasma enzymes was identical with crude LPL activity from adipose tissue. In this publication we report on studies which led to a highly purified LPL from rat adipose tissue. LPL was purified by affinity chromatography on Sepharose 4B containing covalently linked heparin. The preparations obtained were purified 1500-fold. By isoelectric focusing this enzyme showed a single band of activity at pI 4.2. The effect of different concentrations of NaCl, Ca^+ and heparin on the purified enzyme as well as the stability of the enzyme were investigated. These studies were designed to allow more direct comparison of purified plasma and tissue triglyceride lipases.

2. Materials and methods

2.1. *Enzyme assays*

Triglyceride lipase assays were carried out as follows: in a total volume of 1.0 ml each vial contained i) 3.3 nmol of [1-^{14}C]trioleate (7.3×10^4 dpm); ii) 0.76 μmol of unlabeled triolein; iii) 5 mg bovine albumin; iv) 13.4 μl of a 1:100 diluted Triton X-100 solution; v) 50 μl of normal human serum as cofactor; vi) variable amounts of enzyme solution. Final buffer concentrations were 0.2 M Tris–HCl (pH 8.4) with 0.1 M NaCl. Incubations were done in duplicate for 30 min at 27°C. Total lipids were extracted by the method of Dole and Meinertz and ^{14}C-labeled free fatty acids were isolated on an ion exchange resin by a modification [7] of the method of Kelley [8]. Enzyme activity was calculated as nmol FFA/ml/hr. FFA release was linear with time for 1 hr or until 10–12% of the triglyceride was hydrolyzed.

2.2. *Enzyme preparation*

Rat epididymal fat pads were delipidated with cold acetone and ether as previously described [9]. The delipidated powder was then homogenized with sodium barbital (0.005 M, pH 7.4) using a Potter–Elvehjem homogenizer for 2 min at 4°C. The resulting suspension was centrifuged at 800 *g* for 30 min at 4°C. The clear supernatant was decanted and diluted with sodium barbital to a final volume of 100 ml.

Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden) was activated by treatment with cyanogen bromide [10] and heparin was covalently bound to the gel as described by Iverius [11]. The sample (100 ml) was applied to a column of heparin–Sepharose (2.5 × 10 cm) and stepwise elution was carried out with solutions of 0.4 M NaCl (100 ml), 0.75 M NaCl

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Table 1
Summary of the purification steps for rat adipose tissue TGL.

	Volume (ml)	Protein (mg)	Total activity (nmol FFA/hr)	Specific activity (nmol FFA/hr per mg protein)	Purification yield (%)	
Sodium barbital extract[+]	100	127	79 116	620	–	100
Eluate 1[++]	200	126	19 836	160	–	25
Eluate 2[+++]	100	0.250	4 218	16 870	27	5
Eluate 3[++++]	60	0.015	13 680	912 000	1465	17

Data represent enzyme activity in 0.1 M sodium chloride.

[+] Sodium barbital (0.005 M, pH 7.4) extract of delipidated rat epididymal fat pads.
[++] Eluate 1 (100 ml starting buffer (Sodium barbital 0.005 M, pH 7.4) + 100 ml sodium barbital with 0.4 M NaCl) from the heparin–Sepharose column.
[+++] Eluate 2 (0.75 M NaCl. 0.005 M, sodium barbital, pH 7.4) from the heparin–Sepharose column.
[++++] Eluate 3 (1.2 M NaCl. 0.005 M, sodium barbital, pH 7.4) from the heparin–Sepharose column.

(100 ml) and 1.2 M NaCl (60 ml) respectively. Eluates with highest specific activity (table 1) were concentrated by pressure dialysis to a final volume of approximately 60 ml before isoelectric fractionation.

2.3. *Isoelectric focusing and polyacrylamide gel electrophoresis*

The isoelectric focusing column (LKB 8100-10, vol 110 ml) gradient mixing device (LKB 8121) and pH 3–5 ampholine carrier ampholytes were obtained from LKB Producter AB, Stockholm-Bromma 1, Sweden. A dense electrode solution containing 0.2 ml conc. sulfuric acid, 12 g sucrose and 14 ml water was added to the anode at the bottom of the column. A sucrose gradient containing carrier ampholytes was slowly introduced into the column using the gradient device. The dense gradient solution contained 28 g sucrose, 2 ml (40% w/v) carrier ampholytes, and 42 ml water. The light gradient solution contained 0.5 ml carrier ampholytes in 60 ml enzyme solution. Final concentration of carrier ampholytes in the gradient was 1% (w/v). When the column had filled the light electrode solution, consisting of 10 ml NaOH (1% w/v) was added to the cathode at the top of the column. About 2 hr were required to fill the column. A potential of 500 V was applied to the column for 18 hr after which the current dropped from 14 A to 1.2 A. All operations were performed at 2°C. When isoelectric focusing was completed the valve at the bottom of the column was closed and the column was emptied at a flow rate of about 0.5 ml/min. Fractions of approximately 2 ml were collected and the pH of each sample was determined.

Polyacrylamide gel electrophoresis was performed in 5.5 and 3.75% acrylamide respectively. The gels were prepared with and without 1% dodecyl sulfate using Tris–HCl buffer. Protein bands were visualized by Coomassie blue staining [12]. Protein was measured by the method of Lowry et al. with bovine albumin as standard [13].

3. Results

Most of the protein from the epididymal fat pads did not bind to the heparin–Sepharose column and eluted during application of the sample in sodium barbital as summarized in table 1. By stepwise elution with increasing amounts of NaCl in sodium barbital buffer lipoprotein lipase eluted at 1.2 M NaCl. The final specific activity was 912×10^3 nmol FFA/hr per mg protein, representing approx. 1500-fold purification from crude sodium barbital extracts of delipidated rat epididymal fat pads. Polyacrylamide gel electrophoresis was performed in order to both visualize protein bands and determine enzyme activity of the gel. While only one major band is seen in the separation gel (fig. 1) assay of gel slices revealed enzymatic activity at sites corresponding to this band as well as in the stacking gel (fig. 1). This latter activity probably represents some aggregation of the enzyme perhaps occurring during the stacking process. Iso-

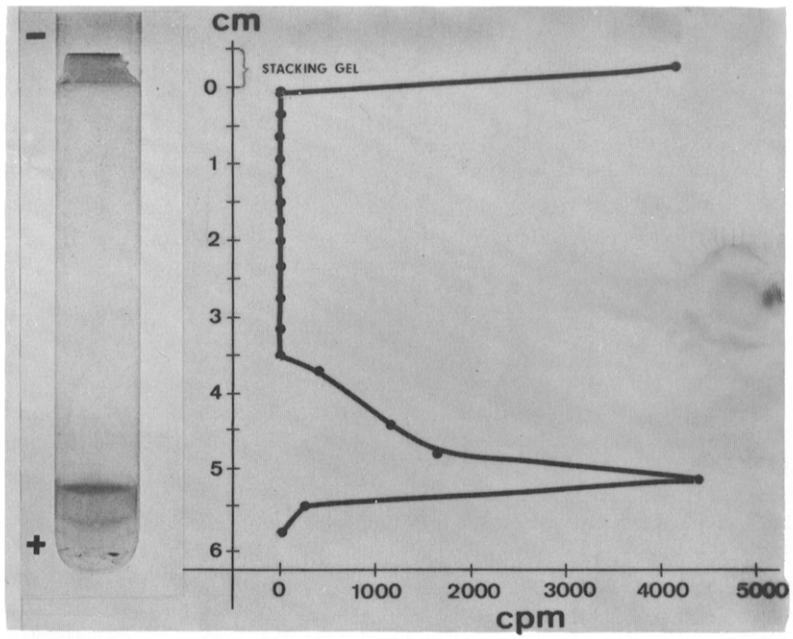

Fig. 1. PA of purified rat adipose tissue LPL (3.75% without SDS) of concentrated fractions with LPL activity obtained from the heparin–Sepharose column. Samples in 1.2 M NaCl were first desalted by passing over a Sephadex G-25 column before application to the gel. A major band is seen in the separation gel with enzyme activity corresponding to this band. With 5.5% acrylamide gel this band moved only half way into the gel and no further bands were visible.

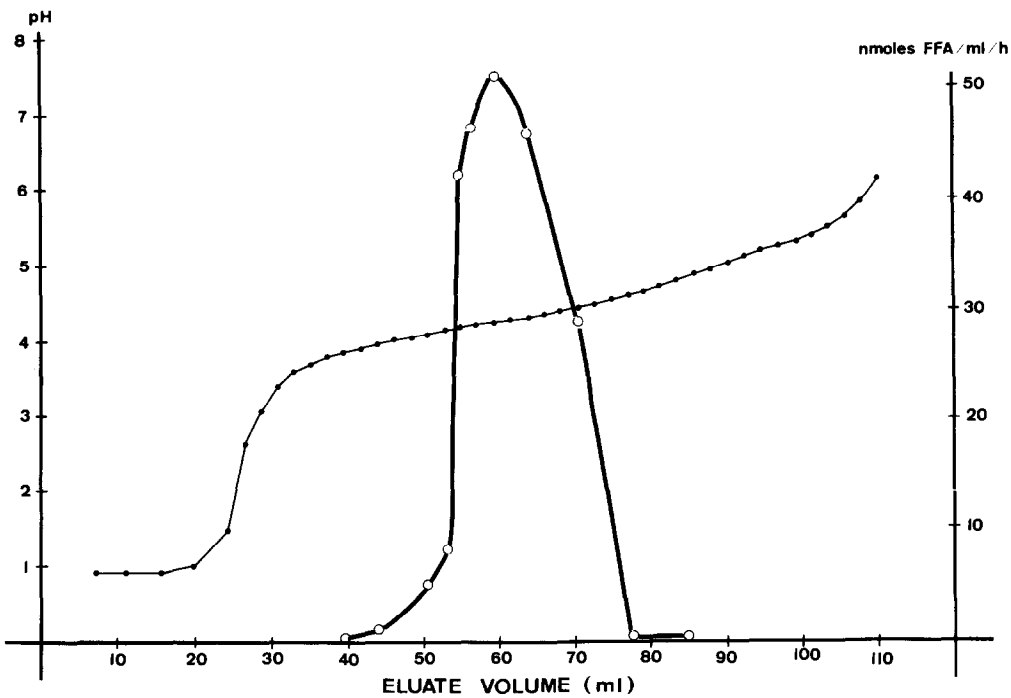

Fig. 2. Isoelectric focusing of purified rat adipose tissue LPL. 60 ml of enzyme solution, obtained from the heparin–Sepharose column in 0.005 M sodium barbital, 1.2 M NaCl were dialyzed against water for 2 hr before application on the column. For further details of the method see the text.

Table 2
The effects of NaCl, $CaCl_2$ and heparin on purified rat adipose tissue TGL.

NaCl		$CaCl_2$		Heparin	
NaCl (M)	nmol FFA/ml/hr	$CaCl_2$ (mM)	nmol FFA/ml/hr	Heparin (μg)	nmol FFA/ml/hr
0	205	0	205	0	203
0.05	263	1	159	1	255
0.10	265	5	148	2.5	223
0.15	226	10	146	5	219
0.25	148	25	60	10	217
0.50	49	50	8	20	200
0.75	39			30	192
1.00	39			50	167
1,50	26				
2.00	26				

electric focusing of the purified enzyme revealed a single band of activity at pI 4.2 (fig. 2). The effects of increasing NaCl, $CaCl_2$ and heparin concentrations on purified rat adipose tissue LPL are shown in table 2. In the absence of NaCl, less activity could be measured, while increasing salt concentrations strongly inhibited the enzyme. $CaCl_2$ already at low levels markedly inhibited purified LPL under the present assay conditions. Low concentrations of heparin showed approx. 10% activation with a decrease in enzyme activity at higher concentrations. The purified enzyme had a half life of approx. 24 hr at 4°C. Stability of the enzyme could best be increased by keeping the purified enzyme in a 1% bovine albumin solution (table 3). With this addition loss of activity after 5 days storage at 4°C was only 50% versus 90% loss without albumin.

4. Discussion

A triglyceride lipase has been highly purified from rat adipose tissue. The purified enzyme required plasma as cofactor for full activity and was strongly inhibited by high sodium chloride, calcium chloride and to some extent by high heparin concentrations. Similar effects of NaCl and heparin have recently been reported for lipoprotein lipase from hen adipose tissue [14].

Triglyceride lipases present in plasma or tissue play an important role in the metabolism of triglycerides. For better understanding of these metabolic processes the various enzymes involved should best be studied in purified form. Several lipases have recently been characterized and purified from post-heparin plasma

Table 3
Summary of experiments for stabilisation of purified lipoprotein lipase.

	Storage after purification					
	0 Days (nmol FFA/ml/hr)	Loss (%)	1 Day (nmol FFA/ml/hr)	Loss (%)	5 Days (nmol FFA/ml/hr)	Loss (%)
Purified enzyme	32.25	0	14.80	54	3.17	90
+ $CaCl_2$ (10^{-6} M)	20.31	37	13.76	32	8.52	58
+ 1% Bovine albumin	30.59	5	23.68	33	16.00	48
+ 1% Bovine albumin + $CaCl_2$ (10^{-6} M)	30.59	5	22.43	27	16.15	47
+ 0.02 M mercaptoethanol	9.24	71	2.44	74	0.93	90

[5, 15–17], milk [18], and tissues [14, 19]. A concept of multiple lipases occurring in post-heparin plasma was established [14] and two lipases with different characteristics have been purified from plasma [5, 15]. While one form of plasma triglyceride lipase [5] had the characteristics of a liver TGL [19], the second form resembled the adipose tissue LPL [15]. Many investigators have used rat adipose tissue as enzyme source for LPL. This study led to a highly purified adipose tissue enzyme which may be used as a model in comparative studies with purified plasma triglyceride lipases.

Acknowledgement

These studies were supported by the Deutsche Forschungsgemeinschaft (Az. Gr. 258/4).

References

[1] Korn, E. (1959) Methods Biochem. Anal. 7, 145.
[2] LaRosa, J.C., Levy, R.I., Herbert, P., Lux, S.E. and Fredrickson, D.S. (1970) Biochem. Biophys. Res. Commun. 41, 57.
[3] Havel, R., Shore, V., Shore, B. and Bier, D. (1970) Circulation 41 Suppl. III, 6.
[4] LaRosa, J.C., Levy, R.I., Windmueller, H.G. and Fredrickson, D.S. (1972) J. Lipid Res. 13, 356.
[5] Greten, H., Walter, B. and Brown, W.V. (1972) FEBS Letters 27, 306.
[6] Greten, H., Brown, W.V. and Steinberg, D., Abstracts of the 9th International Congress of Biochemistry, Stockholm, 1973.
[7] Huttunen, J.K., Ellingboe, J., Pittman, R.L. and Steinberg, D. (1970) Biochim. Biophys. Acta 218, 333.
[8] Kelley, T.F. (1968) J. Lipid Res. 9, 799.
[9] Greten, H., Levy, R.I. and Fredrickson, D.S. (1968) Biochim. Biophys. Acta 164, 185.
[10] Axén, R., Porath, J. and Ernback, S. (1967) Nature 214, 1302.
[11] Iverius, P.H. (1971) Biochem. J. 124, 677.
[12] Rodbard, D. and Chrombach, A. (1971) Anal. Biochem. 40, 95.
[13] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265.
[14] Engelrud, T. (1973) Biochim. Biophys. Acta 296, 124.
[15] Fielding, C.J. (1970) Biochim. Biophys. Acta 206, 109.
[16] Ganesan, D. and Bradford, P.H. (1971) Biochem. Biophys. Res. Commun. 43, 544.
[17] Yasuoka, S. and Fujii, S. (1971) J. Biochem. 70, 937.
[18] Egelrud, T. and Olivecrona, Th. (1972) J. Biol. Chem. 19, 6212.
[19] Assmann, G., Krauss, R.M., Fredrickson, D.S. and Levy, R.I. (1973) J. Biol. Chem. 248, 1992.