THE METABOLISM OF VERY LOW DENSITY LIPOPROTEIN AND CHYLOMICRONS BY PURIFIED LIPOPROTEIN LIPASE FROM RAT HEART AND ADIPOSE TISSUE

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SUMMARY: Crude lipoprotein lipase, extracted from rat adipose tissue or heart acetone-ether powders, was purified about 300 and 350 fold respectively by affinity chromatography. Artifactual increments in the density of very low density lipoprotein, noted after incubation with the crude lipoprotein lipase extract from adipose tissue, were abolished when the purified enzyme was used. Purified enzymes from both tissues showed similar modifications of activity in the presence of activators and inhibitors. The triglyceride moieties of various natural substrates were preferentially hydrolysed in the order Very low density lipoprotein > Serum chylomicrons > Thoracic duct chylomicrons by both enzymes.

INTRODUCTION: The catabolism of serum lipoproteins has generally been studied in vivo, using lipoproteins labelled in the protein (1) or lipid (2,3) moieties. Certain portions of these moieties, however, are now known to undergo free exchange (4-7), making data obtained in vivo difficult to interpret. We therefore undertook studies on the metabolism of discrete lipoprotein species in vitro in the presence of lipoprotein lipase (LPL) and absence of serum. During these investigations we observed artifactual increments in density when very low density lipoprotein (VLDL) was incubated with LPL extracted from rat adipose tissue acetone-ether powders. We also noted that radioactive chylomicrons obtained from the thoracic duct of rats were hydrolysed at a very low rate by purified adipose tissue LPL but proved to be a good substrate for the isolated perfused rat heart (8). Heart and adipose tissue LPL are known to respond in opposite directions to dietary factors (9, 10) and to our knowledge there is no information on the relative activity of the enzymes against their natural substrates. Thus we were prompted to purify LPL from both sources and compare their general properties. The results of this investigation are presented in this report.

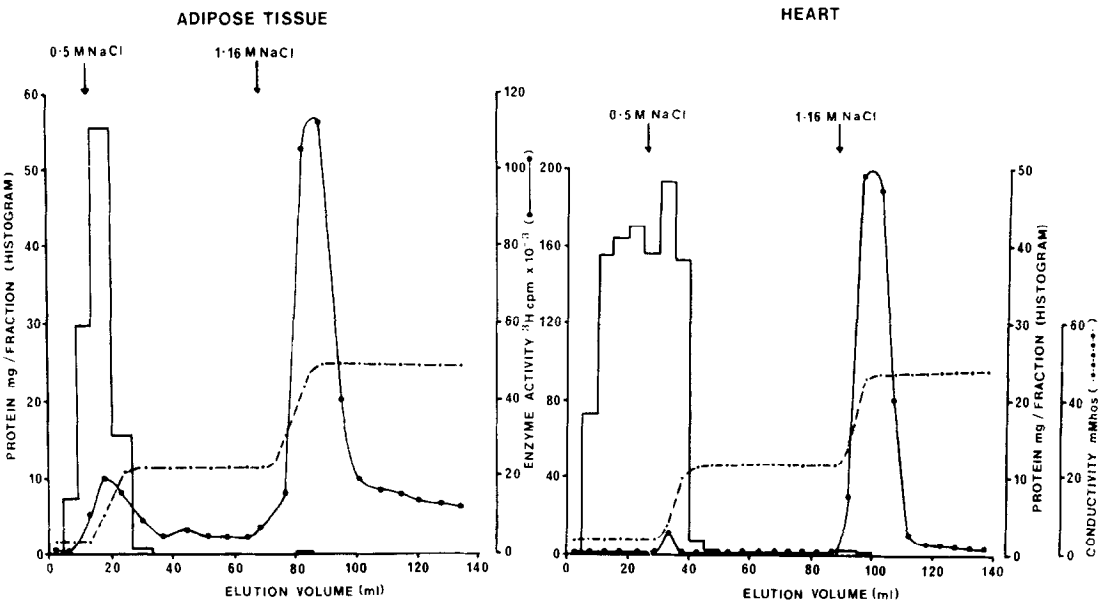

FIG. 1.

The purification of rat adipose tissue and heart lipoprotein lipase by affinity chromatography.

114 mg, (12 ml) of NH_4OH/NH_4Cl extract of rat adipose tissue or 272 mg (24 ml) extract of rat heart acetone-ether powders were applied to 9.5 x 1 cm columns of heparin co-valently linked to Sepharose 4B at a flow rate of 15 ml/h. Non-specifically bound protein was then eluted with 60 ml of 0.005 M veronal buffer pH 7.4 containing 0.5 M NaCl, (flow rate 35 ml/h) and LPL subsequently eluted with 60 ml veronal buffer pH 7.5 containing 1.16 M NaCl.

1 ml aliquots of all fractions were assayed immediately after elution (17) in a medium containing: 28.8 mg Bovine serum albumin, 0.24 ml 1% Triton X-100,3.5 mg Triolein, 1.2ml rat serum, 0.154 M NaCl, 2 mM $CaCl_2$ and 0.1 M Tris-HCl pH 8.6 in a total volume of 8.6 ml. Incubations were for 1 h at 27°.

MATERIALS AND METHODS: LPL was extracted from 0.25 g of rat epididymal fat pad acetone-ether powder (11) with a total of 15 ml 0.05 M NH_4OH/NH_4Cl buffer pH 8.5 at 4° for 40 min. using a close fitting homogeniser. The extract was centrifuged and the supernatant used for subsequent enzyme purification. 1.0g of heart acetone-ether powders was extracted by the same procedure except 30 ml of buffer was used. LPL was purified from the crude extracts by a modification of the procedure of Egelrud and Olivecrona (12) using columns of heparin linked to CNB-activated Sepharose 4B as

described in Fig 1. Adipose tissue LPL was routinely purified 300 fold over the crude NH_4OH/NH_4Cl extract with a 95% yield of enzyme activity. Higher purification (350 fold) but reduced yield (36%) may be obtained by eluting non-specifically bound protein with 0.65 M rather than 0.5 M NaCl. A similar effect has been reported by Greten and Walter (13). Heart LPL was similarly purified by 350 fold with a 95% yield of activity. (Fig 1). This report represents the first purification of LPL from rat heart. Purified LPL from both tissues was unstable, 50% of the activity remaining after 3 h storage in the column eluants at 4°. In contrast LPL in crude heart powder extracts was stable in 1 M NaCl at 4°, (16% loss of activity in 3 h). LPL in crude adipose tissue extracts, however, was unstable, (80% loss of activity in 3 h) under these conditions. Crude adipose tissue LPL and both purified enzymes were stabilized, (46% and 18% loss of activity in 3 h respectively) by addition of glycerol to a final concentration of 10%. Both purified enzymes could be stored in 10% glycerol and 10 mg/ml bovine serum albumin at - 10° for 2 days without substantial loss of activity. Extraction of heart acetone ether powders with 0.005 M veronal buffer pH 7.4 containing 1 M NaCl gave an extract containing 55% more enzyme activity and 30% more protein,resulting in a 1.3 fold greater specific activity than in equivalent NH_4OH/NH_4Cl extracts. Similar observations have been made by Bensadorn et al (14) for extracts of pig adipose tissue. Due to the high protein concentration (17 mg/ml) and the need to dilute and dialyse the high salt extract prior to affinity chromatography this method of extraction was not routinely employed. Both purified heart and adipose tissue were equally stimulated by 9 µg/ml heparin and inhibited by 1 M NaCl, 23 µg/ml heparin, and 10m M $CaCl_2$.

Radioactive rat serum chylomicrons and VLDL were obtained by intravenous injection of 50 µCi [9,10-^{3}H] palmitic acid bound to bovine serum albumin. The rats were exsanguinated 30 min after injection. Radioactive thoracic duct chylomicrons were prepared by administration of 1 mCi [9,10-^{3}H] palmitic acid in 1 ml corn oil by stomach tube to rats whose thoracic ducts had previously been canulated (15).

TABLE I

Distribution of radioactivity from VLDL containing [^{3}H] palmityl glyceride after incubation with crude and purified LPL.

Fraction assayed		C P M x 10^{-3} / flask			
density	lipid	no enzyme	crude LPL		purified LPL
			control	1 M NaCl	
		VLDL			
1.006	glyceride	137	7	7	154
1.006-1.063	glyceride	1	28	35	3
1.063-1.21	glyceride	1	17	7	>1
>1.21	glyceride	3	47	90	>1
>1.21	fatty acids	3	37	21	32
		Triolein			
	glyceride	79	63	68	
	fatty acid	2	17	9	

The triolein assay was described in Fig, 1. Radioactive VLDL was incubated for 2 h at 27° with or without LPL at an NaCl concentration of 0.154 M or 1M as indicated. When VLDL was the substrate Triton X-100, triolein and serum were omitted from the assay medium (8.6ml). After incubation the medium was ultracentrifugally fractionated into the density ranges shown, the lipid moiety extracted, and the triglyceride and fatty acid radioactivity determined.

Chylomicrons were isolated by ultracentrifugation for 1 h at a density of 1.006 and VLDL was subsequently isolated by ultracentrifugation for 18 h at the same density. Both fractions were washed once by ultracentrifugation at the same density. Other lipoprotein fractions were prepared by ultracentrifugation as described by Havel et al (16) using an International B-60 ultracentrifuge and SB 283 rotor. LPL was assayed using [^{3}H] oleyl triolein as substrate (17). Total lipids present after incubations of triolein, VLDL and chylomicrons were extracted (18), partitioned into neutral lipids and free fatty acids (19) and counted. Triglycerides were quantitated by the method of Van Handel and Zilversmit

TABLE 2

The hydrolysis of various substrates by purified rat heart and adipose tissue LPL

Substrate	Triglyceride hydrolysed (μg) heart enzyme	adipose enzyme	Relative * activity
Triolein + serum	169	260	100
Triolein + serum + 1M NaCl	39	78	27 ± 3(4)
Triolein (no serum)	24	30	15 ± 3(4)
VLDL	371	595	247±37(4)
Serum chylomicrons	148	228	76 ± 4 (6)
Thoracic duct chylomicrons	78	96	48 ± 8 (6)

*Value represent the mean ± S.E. relative to the activity of triolein. The means include experiments with LPL from both tissues; number of experiments in parenthesis. The results have been corrected for the initial low fatty acid content of the substrates.

All incubations were performed in duplicate for 1 h at 27° and contained 3.5 mg triglyceride in a total volume of 8.6 ml. The triolein assay was performed as in Fig, 1.

When VLDL and chylomicrons were used, Triton X-100, serum and triolein were omitted from the medium.

(20) and protein by the Biuret method (21) and the procedure of Lowry et al (22).

RESULTS: The effects of incubation of VLDL containing radioactive esterified fatty acid with crude and purified LPL is shown in Table 1. A parallel triolein assay was performed to monitor enzyme activity. After incubation, the medium containing VLDL was ultracentrifugally fractionated into density ranges corresponding to VLDL, LDL and HDL and the radioactivity associated with the glycerides and free fatty acids determined. Incubations with the crude enzyme preparation resulted in the appearance of fatty acids, presumably bound to albumin, in the d>1.21 fraction and a redistribution of the unhydrolysed glycerides into densities corresponding to LDL, HDL and d>1.21. Inhibition of enzyme activity by 50% with 1 M NaCl did not significantly affect the redistribution of VLDL

glycerides which was seen in the absence of the inhibitor. This suggests that non-specific protein binding artifactually increased the density of VLDL. This is borne out by use of the purified enzyme where a similar degree of lipolysis results in the appearance of only free fatty acids in the d>1.21 fraction and a small but significant shift of glyceride radioactivity into the LDL region.

The catabolism of various natural substrates by purified heart and adipose tissue LPL was then investigated. The results shown in Table 2 demonstrate that both enzymes require the presence of serum for full activity against the synthetic triolein substrate. Both enzymes hydrolyse the triglyceride moieties of VLDL, serum chylomicrons and chylomicrons from the thoracic duct. There was little difference between the activities of each enzyme towards a particular substrate but both enzymes showed a well defined and similar order of preference for the substrates. VLDL proved the best substrate for both enzymes and thoracic duct chylomicrons the worst. This observation is more clearly seen if the activities of both enzymes towards the lipoprotein substrates are related to a constant triolein activity of 100. The small standard errors show that there is a very significant difference between the extent of hydrolysis of the various substrates but very little difference in the activity of each enzyme to one particular substrate.

DISCUSSION: This report raises three important points. Firstly, purified heart and adipose tissue LPL exhibit well defined preferences for natural substrates reflecting their proportions of activator peptide. Our finding that serum chylomicrons are hydrolysed to a greater extent than thoracic duct chylomicrons can be explained by the observation of Havel et al. (23) that duct chylomicrons acquire activator peptide on passing into the serum. The greater extent of VLDL hydrolysis probably reflects the higher protein and hence activator content of this substrate. Secondly, purified LPL from both tissues have similar properties with respect to activators and inhibitors and exhibit the same relative activities with natural substrates. It is possible that the

enzymes represent similar molecular species despite their opposite responses to dietary factors. Thirdly, studies of lipoprotein metabolism in vitro with tissue LPL must utilize purified enzymes if artifactual increments in density of the substrate and possible products are to be avoided.

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