ASSOCIATION OF LIPOPROTEIN LIPASE WITH VERY LOW DENSITY LIPOPROTEIN REMNANTS

Influence of high density lipoproteins

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1. Introduction

Hydrolysis of triglycerides transported by plasma chylomicrons and very low density lipoprotein (VLDL) occurs in at least two steps [1]. First, chylomicrons and VLDL are converted to triglyceride-depleted remnant particles at the capillary endothelial cell surface by the action of lipoprotein lipase (EC 3.1.1.3). The second process, thought to occur in the liver, involves the uptake and modification of remnant particles by the action of a hepatic lipase [1]. For maximal activity, extrahepatic lipoprotein lipase requires a specific protein activator, apolipoprotein C-II (apoC-II), which is a constituent of VLDL and of high density lipoproteins (HDL) [2–6]. As they enter the circulation, nascent chylomicrons and VLDL acquire apoC-II from HDL; during lipolysis apoC-II returns to HDL.

The mechanism by which apoC-II activates lipoprotein lipase is not well understood. ApoC-II contains 78 amino acid residues, the sequence of which is known [7]. Studies with both native [8,9] and synthetic [9] fragments of apoC-II have demonstrated that the residues between 55 and 78 contain the minimal sequence for activation of the enzyme. Rat heart lipoprotein lipase and apoC-II has been shown [10] by monolayer techniques to associate in a 1:1 complex with a $K_d < 10^{-8}$ M^{-1}. $K_d = 10^{-10}$ M^{-1} was reported [11]. ApoC-II and lipoprotein lipase have also been shown to associate at a monolayer surface in the absence of lipid [12]. With $K_d = 10^{-8}$–10^{-10} M^{-1} it is reasonable to assume that lipoprotein lipase binds to chylomicrons and VLDL, and that the amount of lipoprotein lipase bound per lipoprotein particle correlates directly with the apoC-II content. Here we have utilized ^{125}I-labeled lipoprotein lipase to demonstrate that the enzyme is associated with VLDL-remnants in the absence of HDL. When HDL was included in the reaction mixture, there was a 4-fold decrease in the amount of apoC-II and a 10-fold decrease in ^{125}I-labeled lipoprotein lipase associated with VLDL remnants.

2. Materials and methods

Lipoproteins were isolated from plasma of normal fasting individuals by ultracentrifugation in solutions of KBr. The density ranges for each lipoprotein were as follows: VLDL, $d < 1.006$; low density lipoproteins (LDL), $d = 1.020$–1.050; and HDL, $d = 1.063$–1.210. The centrifugation was performed in a Beckman Model L2-65 (Spinco Div., Palo Alto, CA) with a type 50.2 Ti rotor operated at 4.8×10^4 rev./min for 22 h at 8°C. Lipoproteins were dialyzed against 0.9% NaCl, 0.001 M EDTA, 0.05 M Tris–HCl (pH 7.4).

ApoC-II was isolated from VLDL as in [7]. The isolated protein was homogeneous as determined by isoelectric focusing [13] and by amino acid analysis. ApoC-II was quantitated by radioimmunoassay as in [14].

Bovine milk lipoprotein lipase was purified to homogeneity by affinity chromatography on heparin–Sepharose as in [15]. The isolated enzyme had spec. act. 36 mmol fatty acids released.h^{-1}.mg $protein^{-1}$ using glycerol [^{14}C]triolein emulsified with 5% gum arabic as substrate [16]. Iodination of the lipase was performed by a modified procedure of [17] as reported [18]. Lipoprotein lipase was incubated at −10°C in 1 ml 0.10 M Tris–HCl (pH 9.6) containing 20% glycerol. One mol equivalent of iodine was added and after 30 min at −10°C the enzyme was dialyzed overnight against 0.05 M Tris–HCl (pH 7.6) containing 10% glycerol; 33% of the ^{125}I was not precipitated by trichloroacetic acid (10%). Iodination did not alter the specific activity of the enzyme. Lipase was stored at −20°C in 50% glycerol. The iodinated enzyme contained 2.7×10^4 precipitable cpm/μg lipase (0.45 iodide/lipase).

Lipoprotein protein concentrations were determined by the method in [19]. Phospholipid phosphorus was determined by the procedure in [20]. Lipoprotein cholesterol and triglyceride were quantitated by auto-analyzer techniques [21]. Fatty acids were extracted by the procedure [22] and were analyzed by gas chromatography [23]. ^{125}I radioactivity was determined in a Beckman Biogamma scintillation spectrometer.

3. Results

Human plasma VLDL were incubated with ^{125}I-labeled lipoprotein lipase; VLDL-remnants were isolated from the incubation mixture by ultracentrifugal flotation at $d = 1.063$. As is shown in table 1, ~25% of the radioactivity was in the $d = 1.063$ top, indicating that lipoprotein lipase associates with VLDL-remnants. Lipolysis was also performed in the presence of HDL under conditions in which the ratio of HDL to VLDL was such that the apoC-II content of each lipoprotein was equal, thus eliminating a net transfer of apoC-II. In the presence of HDL, the amount of ^{125}I-labeled lipoprotein lipase associated with VLDL remnants was 10% of that associated with remnants in the absence of HDL. Moreover, the amount of radioactivity in the $d = 1.063$ infranatant was identical to that which occurred when only HDL and lipoprotein lipase were incubated. To determine

Table 1
Ultracentrifugal distribution of ^{125}I-labeled lipoprotein lipase after in vitro lipolysis of human plasma VLDL[a]

Addition	Total cpm[b]		Recovery of ^{125}I (% total)
	Top	Infra-natant	
VLDL	3270	9343	74.3
VLDL + HDL	260	12 128	74.1
HDL	102	11 824	81.0
LDL	279	11 006	72.2

[a] Incubations were performed in 17 × 100 mm polypropylene tubes (Falcon Plastics) in 5.0 ml total vol. of a standard buffer containing 0.9% NaCl, 0.05 M Tris–HCl (pH 7.8). Each incubation mixture contained 75 mg fatty-acid free bovine serum albumin (Sigma) and the indicated human plasma lipoproteins as follows: VLDL (1.11 mg protein, 77 μg apoC-II, 7.0 mg triglyceride, 3 μmol phospholipid); HDL (7.6 mg protein, 100 μg apoC-II, 5.2 μmol phospholipid); and LDL (1.6 mg protein, 1.6 μmol phospholipid). Each reaction was initiated by the addition of 2 μg (36 pmol) bovine milk ^{125}I-labeled lipoprotein lipase (2.695×10^4 precipitable cpm/μg). After incubation for 1 h at 37°C with shaking, the mixtures were rapidly cooled to 0°C and the following samples were taken for analysis: 1 ml for fatty acid analysis, 1.5 ml was layered over 3.5 ml of standard buffer containing KBr at $d = 1.063$ and ultracentrifuged as in section 2; 1.0 ml was chromatographed on Bio Gel A5m (fig.1); and 1.5 ml was counted to determine the percent recovery of ^{125}I-labeled lipoprotein lipase

[b] After ultracentrifugation of 1.5 ml of the incubation mixture, the top 1 ml containing the VLDL-remnants, and the infranatant 4 ml were removed, dialyzed and the ^{125}I determined

if binding of ^{125}I-labeled lipoprotein lipase is specific for VLDL remnants, LDL were incubated with ^{125}I-labeled lipoprotein lipase, and the LDL were reisolated by ultracentrifugation at $d = 1.063$. As is shown in table 1, most of the radioactivity was in the $d = 1.063$ infranatant, indicating that lipoprotein lipase does not bind to LDL.

We next attempted to correlate the amount of ^{125}I-labeled lipoprotein lipase associated with the VLDL remnants with the amount of apoC-II. VLDL remnants produced in the absence of HDL contained 1200 pmol apoC-II (table 2); those formed in the presence of HDL contained 340 pmol. Based on the ^{125}I specific activity of lipoprotein lipase, the molar ratio of apoC-II to lipoprotein lipase was 545 in the absence of HDL and 1700 in the presence of HDL.

Table 2
Ultracentrifugal fate of VLDL constituents during in vitro lipolysis by lipoprotein lipase (LpL)[a]

Addition	ApoC-II (pmol)	^{125}I-LpL (pmol)	phospholipid[b] (nmol)	ApoC-II / LpL	Phospholipid[c] / ApoC-II
VLDL	1200 (45%)[b]	2.2	706	545	588
VLDL + HDL	340 (13%)[b]	0.2	413	1700	1214

[a] The top fractions (VLDL remnants) described in table 1 were analyzed for apoC-II content by the radioimmunoassay procedure in [14]. ^{125}I-labeled LpL was determined from the data in table 1, based on the radioactivity of the enzyme (2.695×10^4 cpm/μg). Phospholipid was measured by the method in [20]. Recovery of phospholipid was 69–78%; recovery of apoC-II varied from 75–121%

[b] The number in parentheses represents the % recovery of apoC-II; 2674 pmol total apoC-II were present in 1.5 ml of the original incubation solution

[c] The phospholipid/apoC-II molar ratio of the initial VLDL was 352

The addition of HDL also caused loss of phospholipid from VLDL remnants (table 2), a result consistent with other reports [1]. The molar ratio of phospholipid to apoC-II was 1200 in the incubation mixture containing HDL relative to 590 in the reaction containing only VLDL. To rule out the possibility that the differences observed in tables 1 and 2 are due to HDL-dependent differences in the extent of triglyceride hydrolysis, the amount of fatty acids released in each of the incubation mixtures was determined. As is shown in table 3, the amount of each of the fatty acids released per ml incubation mixture was approximately the same, indicating that the extent of hydrolysis is not influenced by HDL.

The above results utilizing ultracentrifugal flotation methods to isolate VLDL-remnants suggest that the amount of lipoprotein lipase associated with remnants is dependent on the presence of HDL. To establish that the results are independent of the method of isolation of remnants, the incubation mixtures were also fractionated on a column of Bio Gel A5m; the conditions of column chromatography also avoid the high salt utilized in ultracentrifugal flotation. Figure 1a shows that in the absence of HDL, >90% of the ^{125}I-labeled lipoprotein lipase was associated with VLDL remnants, which eluted in the void volume of the column. In the presence of HDL, only 10% of the ^{125}I-labeled radioactivity was remnant-bound. In the later experiment, most of the enzyme was present in fractions which eluted between HDL and albumin.

4. Discussion

Results of this investigation demonstrate that lipoprotein lipase associated with VLDL remnants produced during VLDL catabolism in vitro. The

Table 3
Fatty acids released after in vitro lipolysis of human plasma very low density lipoproteins[a]

Addition	Fatty acid released/ml incubation mixture (μg)				
	16:0	16:1	18:0	18:1	18:2
VLDL	240	28	45	500	244
VLDL + HDL	288	30	42	464	222

[a] One ml of the appropriate incubation mixture described in table 1 was extracted by the procedure in [22]; the isolated fatty acids were analyzed by gas chromatography [23]. The values represent the μg fatty acids/ml of each incubation mixture

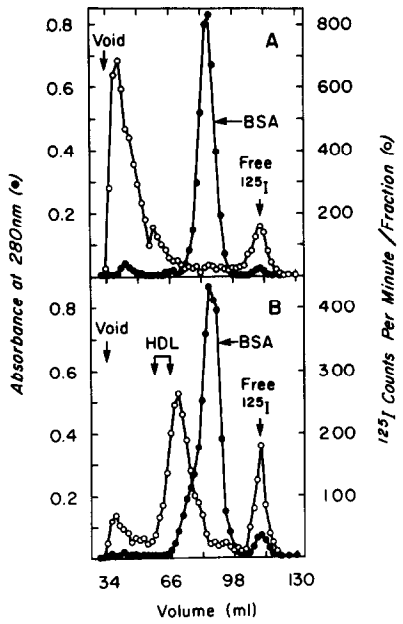

Fig.1. Chromatography of the reaction mixtures on Bio Gel A5m. After in vitro lipolysis in the absence (A) or presence (B) of HDL, 1.0 ml of the incubations (table 1) containing VLDL or VLDL + HDL were chromatographed on a column (1.4 × 87 cm) of Bio Gel A5m equilibrated in 50 mM Tris–HCl (pH 7.8). The flow rate was 30 ml/h and 1.0 ml fractions were collected. The % recovery of ^{125}I activity was: A, 51%; B, 70%. HDL chromatographed on the same column eluted between the arrows.

amount of remnant-associated lipase is reduced by the addition of HDL to the incubation mixture. Furthermore, the amounts of apoC-II and phospholipid in VLDL remnants are also decreased when HDL are present. Whether the HDL-dependent reduction in the amount of lipoprotein lipase bound to VLDL remnants is due to decreased apoC-II and/or to differences in lipid composition or structure remains to be determined. HDL do not, however, influence the amount of fatty acid released during hydrolysis (table 3). As is shown in fig.1, ^{125}I-labeled lipoprotein lipase elutes with VLDL-remnants in the absence of HDL, but dissociates from the remnant particles when HDL are included in the incubation. The dissociated enzyme occurs in a Bio Gel A5m fraction which elutes between that containing HDL and that containing albumin, suggesting that lipoprotein lipase does not bind to HDL. This conclusion is also supported by the findings [24,25] that lipoprotein lipase hydrolyzes phospholipid of chylomicrons and VLDL, but not those of HDL.

Our finding that the amount of remnant-associated apoC-II is determined by HDL is inconsistent with that in [26], that loss of apoC peptides during hydrolysis of rat VLDL catalyzed by bovine milk lipoprotein lipase was independent of the presence of serum in the incubation mixture. It was suggested [26] that apoCs dissociate from VLDL during lipolysis even in the absence of an acceptor (HDL) for the apoC peptides. Loss of apoC peptides was measured [26] by utilizing VLDL which had been labeled with ^{125}I-labeled apoCs. Thus, loss of both apoC-III and apoC-II was followed. In our study, we measured the distribution of apoC-II only. Moreover, the concentrations of VLDL and HDL were chosen such that there was an equivalent amount of apoC-II in each lipoprotein, discouraging a concentration-dependent redistribution of apoC-II.

The data reported herein are of interest since they relate to lipoprotein catabolism in vivo. Under normal conditions, lipoprotein lipase is tightly bound to heparin-like molecules at the capillary endothelial cell surface [1]. Is the release of the enzyme from the endothelial surface requisite for triglyceride catabolism? The studies [27] suggest that enzyme release is unnecessary: the rates of hydrolysis catalyzed by membrane-supported and by solubilized heart lipoprotein lipase are nearly identical. Moreover, addition of VLDL to cultured heart cells was shown [28] not to release bound lipoprotein lipase. However, chylomicron remnants produced during chylomicron catabolism in the supra-diaphragmatic rat was reported [29] to contain lipoprotein lipase activity, suggesting that substrate releases the enzyme from the cell surface. The present findings have not resolved the question, but they demonstrate for the first time that purified lipoprotein lipase binds to VLDL-remnants and that the amount bound is determined by HDL.

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