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Hydrolysis of Human Plasma High Density Lipoprotein$_2$-Phospholipids and Triglycerides by Hepatic Lipase

Kohji Shirai, Roger L. Barnhart and Richard L. Jackson
Division of Lipoprotein Research, Departments of Pharmacology
and Cell Biophysics, Biological Chemistry and Medicine
University of Cincinnati College of Medicine
Cincinnati, OH 45267

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SUMMARY

To determine the physiological role of hepatic lipase in lipoprotein metabolism, human plasma very low (VLDL), low (LDL) or high (HDL$_2$ and HDL$_3$) density lipoproteins were labeled with [^{14}C] triolein or [^{14}C] dipalmitoyl phosphatidylcholine and the rates of lipid hydrolysis were determined using human heparin-releasable hepatic lipase. Compared to LDL and HDL$_3$, HDL$_2$ phospholipids were the preferred substrate for hepatic lipase; 17% of HDL$_2$ phospholipids were hydrolyzed. [^{14}C] Triolein-labeled HDL$_2$ were also hydrolyzed by hepatic lipase. However, purified bovine milk lipoprotein lipase had negligible activity on HDL$_2$-triglycerides. After incubation of HDL$_2$ with hepatic lipase, there was a decrease in the size of the particle as demonstrated by gel filtration and an increase in the density as shown by zonal ultracentrifugation. These results suggest that hepatic lipase may play a role in the interconversion of HDL subfractions.

INTRODUCTION

Intravenous administration of heparin into man releases at least two distinct lipase activities. One enzyme, lipoprotein lipase, is located at the endothelial surface of extrahepatic cells, requires apolipoprotein C-II for maximal activity, is inhibited by 1 M NaCl and hydrolyzes chylomicron and VLDL triglycerides (Ref. 1, for review). A second enzyme, termed hepatic lipase, does not require a protein cofactor, is not inhibited by NaCl and is located on surface of the liver endothelial cells (2). The metabolic function of hepatic lipase in lipoprotein metabolism is not well understood. The enzyme may take part in the catabolism of remnantsof triglyceride-rich lipoproteins (1,3,4). In addition, recent studies suggest that hepatic lipase may play a role in HDL metabolism in the rat (5,6). Jansen et al. (5) and Kuusi et al. (6) showed that when the activity of hepatic lipase was blocked by the intravenous injection of antiserum prepared against hepatic lipase, there was an accumulation of HDL$_2$ cholesterol and phospholipid in the perfusate.

Abbreviations: VLDL (very low), LDL (low) and HDL (high) density lipoproteins.

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The role of hepatic lipase in the metabolism of human lipoproteins is based on clinical correlations. Kuusi et al. (7) have shown that there is an inverse correlation between the activity of hepatic lipase of post-heparin plasma and HDL_2 cholesterol, phospholipid and protein concentrations; no correlation existed between HDL_3 lipids and hepatic lipase. The purpose of the present study was to test these clinical correlations in an in vitro system consisting of purified human hepatic lipase and lipoproteins. We conclude from these studies that HDL_2 are the preferred substrate for hepatic lipase.

MATERIALS AND METHODS

Purification of hepatic lipase and lipoprotein lipase. Hepatic lipase was purified from human post-heparin plasma by affinity chromatography on heparin-Sepharose as described by Iverius and Östlund-Lindqvist (8). The fractions corresponding to hepatic lipase were dialyzed against ammonium sulfate (60%). The resultant precipitate was dissolved in 50% glycerol, 10 mM Tris-HCl, pH 7.4, dialyzed against the same buffer, and stored at -70°C. Hepatic lipase activity was determined using [^{14}C] triolein as substrate. The standard assay mixture contained 0.378 µmol triolein (Sigma), 0.067 µCi tri[^{14}C] oleoyl glycerol (New England Nuclear, specific activity = 50 mCi/mmol), 2% fatty-acid free bovine serum albumin (Fraction V, Sigma), 0.02% Triton X-100, 0.1 M Tris-HCl, pH 8.0 and enzyme protein in a final volume of 0.25 ml. Released [^{14}C] oleic acid was extracted by the method of Belfrage and Vaughan (9). The specific activity of the purified enzyme was 750 mU/mg protein; 1 mU equals the release of 1 nmol free fatty acid/min in the standard assay.

Lipoprotein lipase was purified from bovine milk by affinity chromatography on heparin-Sepharose as described previously (10). The purified enzyme had a specific activity of 350 µmol free fatty acid released/min/mg protein using [^{14}C] triolein as substrate, as described by Matsuoka et al. (10).

Preparation of lipoproteins. VLDL, d <1.006 g/ml; LDL, d= 1.020-1.050 g/ml; and total HDL, d= 1.063-1.210 g/ml were isolated from plasma of normal fasting individuals by ultracentrifugation in salt solutions of KBr. Centrifugation was performed in a Beckman Model L5-65 with a type 50.2 Ti rotor operated at 4.8 x 10^4 rpm for 22 h at 8°C. Lipoproteins were dialyzed against a standard buffer containing 10 mM Tris-HCl, pH 7.4, 1 mM EDTA and 0.01% NaN_3. Total HDL were further fractionated into HDL_2 and HDL_3 by zonal ultracentrifugation in a salt gradient of NaBr (d= 1.00-1.40 g/ml) as described by Patsch et al. (11). Zonal ultracentrifugation was performed at 41,000 rpm and 10°C for 22 h in a Beckman Ti-14 zonal rotor using a L5-65 ultracentrifuge.

Preparation of [^{14}C] triolein-labeled VLDL and HDL_2. VLDL were labeled with [^{14}C] triolein as described by Fielding (12). Tri[1-^{14}C] oleoyl glycerol (10 µCi, specific activity 50 mCi/mmol, New England Nuclear) was dissolved in 1 ml of dimethylsulfoxide, diluted to 4 ml with standard buffer and then VLDL (40 mg triglyceride in 10 ml of standard buffer) were added. After incubation for 3 h at 37°C, the reaction mixture was dialyzed against the standard buffer and the VLDL were reisolated by ultracentrifugation; [^{14}C] triolein-labeled VLDL contained 1.5 x 10^5 cpm/mg triglyceride.

[^{14}C] Triolein-labeled HDL_2 were prepared using [^{14}C] triolein-labeled VLDL and a purified lipid transfer protein which was isolated from human lipoprotein-free plasma as reported previously (13). HDL_2 (2 mg triglyceride), [^{14}C] triolein-labeled VLDL (20 mg triglyceride, 3 x 10^6 total cpm) and lipid transfer protein (1 mg) in a final volume of 15 ml of standard buffer were incubated for 3 h at 37°C. The lipoprotein mixture was then fractionated on a column (1.8 x 80 cm) of Bio-Gel A5m equilibrated with standard buffer.

The fractions corresponding to HDL_2 were pooled and HDL_2 were reisolated by ultracentrifugation (d 1.063-1.210 g/ml); [^{14}C] triolein-labeled HDL_2 contained 6.6 x 10^4 cpm/mg triglyceride, corresponding to the exchange of 50% of HDL_2-triglyceride for VLDL-triglycerides.

Preparation of [^{14}C] dipalmitoyl phosphatidylcholine-labeled LDL, HDL_2 and HDL_3. Dipalmitoyl phosphatidylcholine (20 mg) and 30 μCi of di[^{14}C] palmitoyl phosphatidylcholine (specific activity 100 mCi/mmol, New England Nuclear) were dissolved in chloroform and evaporated in vacuo. Standard buffer was added to the lipid to give 6 mg phospholipid/ml and vesicles were prepared by sonication at 42 °C for 15 min using a Heat Systems Ultrasonics, Inc., Cell Disruptor (Model W-225R). [^{14}C] Dipalmitoyl phosphatidylcholine was incorporated into LDL by using the radiolabeled phospholipid vesicles and bovine liver phospholipid exchange protein as described previously (14); [^{14}C] dipalmitoyl phosphatidylcholine-labeled LDL contained 1.2 x 10^6 cpm/mg phospholipid. [^{14}C] Phospholipid was incorporated into HDL_2 and HDL_3 using [^{14}C] dipalmitoyl phosphatidylcholine-labeled LDL and bovine liver phospholipid exchange protein. The incubation mixtures contained either HDL_2 or HDL_3 (4 mg phospholipid), [^{14}C] dipalmitoyl phosphatidylcholine-labeled LDL (1 mg phospholipid, 1.2 x 10^6 cpm total), 0.2% fatty acid-free bovine serum albumin, bovine liver phospholipid exchange protein (50 μg) in 5 ml of standard buffer. After 1 h at 37 °C, the incubation mixtures were applied to a column (1.6 x 80 cm) of Bio-Gel A5m equilibrated with standard buffer. The fractions corresponding to HDL_2 or HDL_3 were pooled and the lipoproteins were reisolated by ultracentrifugation between d = 1.063-1.210 g/ml. [^{14}C] Dipalmitoyl phosphatidylcholine-labeled HDL_2 and HDL_3 contained 1.3 x 10^5 and 1.5 x 10^5 cpm/mg phospholipid, respectively.

Other methods. [^{14}C] Oleic acid which was released from [^{14}C] triolein-labeled lipoproteins was extracted by the method of Belfrage and Vaughan (9). The release of [^{14}C] lysopalmitoyl phosphatidylcholine from radiolabeled lipoproteins was determined as follows: To the incubation mixture (0.25 ml) was added 0.25 ml H_2O, 1.25 ml MeOH and 0.625 ml $CHCl_3$. After shaking at 0 °C for 30 min, 0.625 ml $CHCl_3$ and 0.75 ml of H_2O were added; the samples were shaken at 0 °C for 30 min. After centrifugation, the $CHCl_3$ phase was removed and evaporated to dryness with nitrogen. The lipids were redissolved in $CHCl_3$ and applied to silica gel 60 (EM Reagents). The thin layer plates (0.25 mm) were developed in $CHCl_3$:MeOH:water (70:30:4); lipids were visualized with iodine vapor. The lipids were scraped from the plates and radioactivity was determined. Protein was determined according to Lowry et al. (15), using bovine serum albumin as standard. Phospholipid phosphorus was measured by the method of Bartlett (16).

RESULTS

Hydrolysis of HDL and LDL phospholipids. To determine the specificity of hepatic lipase for human lipoprotein phospholipids, HDL_2, HDL_3 and LDL were radiolabeled with [^{14}C] dipalmitoyl phosphatidylcholine and the rate of hydrolysis by hepatic lipase was measured (Fig. 1). The hydrolysis of [^{14}C] dipalmitoyl phosphatidylcholine in HDL_2 was 11% in 4 h, whereas the hydrolysis of radiolabeled phospholipid in HDL_3 and LDL was only 3.5% and 1.2%, respectively. To obtain the kinetic parameters K_m and V_{max}, rates of hydrolysis of phospholipid by hepatic lipase were measured at various concentrations of HDL_2 and HDL_3 phospholipids. As shown in Fig. 2-A, the rate of phospholipid hydrolysis increased as the concentration of lipoprotein increased up to 0.6 mM

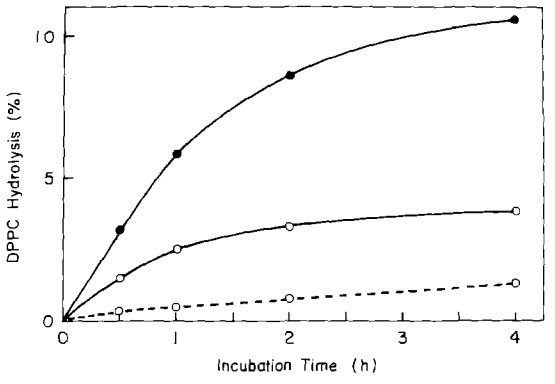


FIGURE 1. Hydrolysis of [^{14}C] dipalmitoyl phosphatidylcholine (DPPC)-labeled HDL_2, HDL_3 and LDL_1 by hepatic lipase. The reaction mixtures contained 100 µg of phospholipid of [^{14}C] DPPC-labeled HDL_2 (●–●), HDL_3 (o–o) or LDL (o---o), 2% fatty acid-free bovine serum albumin, 0.1 M Tris-HCl, pH 8.0 and 30 mU of hepatic lipase in a final volume of 0.25 ml. At the indicated times, enzyme reactions were terminated with $CHCl_3$:MeOH and the released [^{14}C] lysopalmitoyl phosphatidylcholine was determined as described in Materials and Methods.

phospholipid; the Lineweaver-Burk double reciprocal plots of these data are shown in Fig. 2-B. The apparent value of Michaeli's constants (K_m) for HDL_2 and HDL_3 phospholipids were 0.25 mM and 0.45 mM, respectively. The value of maximal reaction velocity (V_{max}) for HDL_2 phospholipids was 1.5 times greater than that for HDL_3 phospholipids.

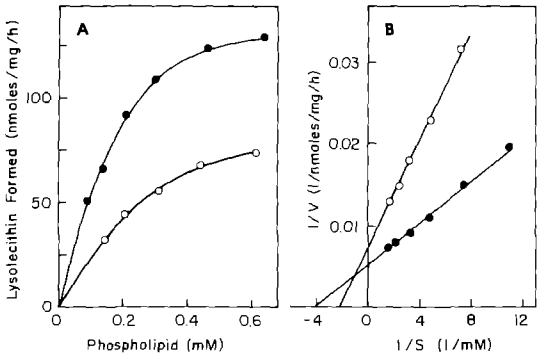


FIGURE 2. (A) Effect of substrate concentration on hepatic lipase activity. Each reaction mixture contained the indicated amount of total phospholipid of [^{14}C] dipalmitoyl phosphatidylcholine-labeled HDL_2 (●–●) or HDL_3 (o–o), 2% fatty acid-free bovine serum albumin, 0.1 M Tris-HCl, pH 8.0 and 30 mU of hepatic lipase in a total volume of 0.25 ml. After incubation for 1 h at 37 °C, enzyme reactions were terminated with $CHCl_3$:MeOH and [^{14}C] lysopalmitoyl phosphatidylcholine was determined as described in Materials and Methods. (B) Lineweaver-Burk double reciprocal plots of the kinetic data in (A). Reaction velocities [V] were determined at varying substrate concentrations [S] of HDL_2 (●–●) on HDL_3 (o–o).

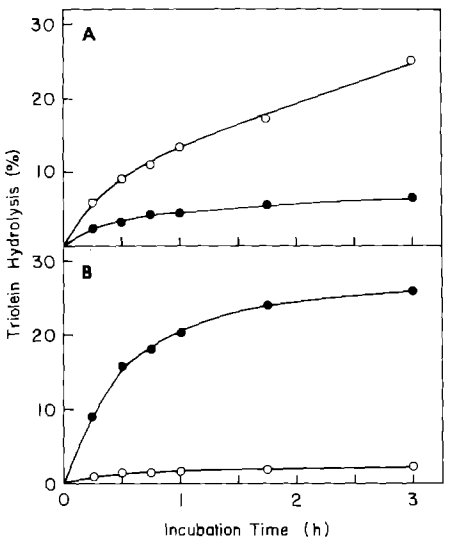
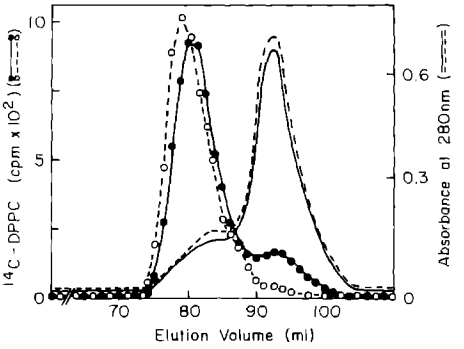


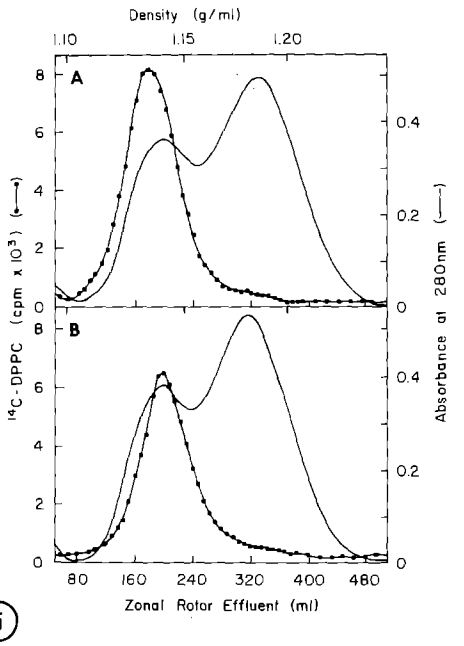
FIGURE 3. (A) Hydrolysis of [^{14}C] triolein-labeled VLDL by hepatic lipase and lipoprotein lipase. Each reaction mixture contained [^{14}C] triolein-labeled VLDL (0.2 mM with respect to triglyceride), 2% fatty acid-free albumin, 0.1 M Tris-HCl, pH 8.0, 0.15 M NaCl and either 12 mU of hepatic lipase (●–●) or 25 mU of lipoprotein lipase (o–o). At the indicated times, enzyme reactions were terminated and released [^{14}C] oleic acids were extracted as described in Materials and Methods. (B) Hydrolysis of [^{14}C] triolein-labeled HDL_2 by hepatic lipase and lipoprotein lipase. The reaction mixtures were the same as described in (A), with the exception that the concentration of HDL_2-triglyceride was 0.1 mM; the amount of hepatic lipase and lipoprotein lipase was also the same.

Hydrolysis of VLDL and HDL_2 triglycerides. From the data described above, we conclude that hepatic lipase preferentially hydrolyzes HDL_2 phospholipids. In contrast to hepatic lipase, lipoprotein lipase has little if any activity on HDL phospholipids (17). It was also of interest to compare the two lipolytic enzymes for their ability to hydrolyze HDL_2 triglycerides. As shown in Fig. 3-B, hepatic lipase hydrolyzes not only HDL_2 phospholipids but also HDL_2 triglycerides. Furthermore, the hydrolysis of [^{14}C] triolein-labeled HDL_2 by hepatic lipase (12 mU) was 10-fold greater than with lipoprotein lipase (25 mU). In contrast to the results with HDL_2, the rate of hydrolysis of VLDL-triglycerides (using the same amount of hepatic and lipoprotein lipase as with HDL_2) was greater with lipoprotein lipase (Fig. 3-A).

Characterization of HDL_2 after incubation with hepatic lipase. [^{14}C] Dipalmitoyl phosphatidylcholine-labeled HDL_2 was incubated with hepatic lipase as described in Fig. 4. After incubation for 6 h, 17% of the radiolabeled phospholipid was hydrolyzed. Phospholipid mass determination showed that 15% of the total phospholipid was



(4)



(5)

FIGURE 4. Chromatography on Bio-Gel A5m of HDL_2 and HDL_2 incubated with hepatic lipase. [^{14}C] Dipalmitoyl phosphatidylcholine-labeled HDL_2 (0.5 mg total phospholipid; 7.5 x 10^4 cpm total), 2% fatty acid-free bovine serum albumin, 0.1 M Tris-HCl, pH 8.0 and either minus (o---o) or plus (●–●) hepatic lipase (420 mU) in a total volume of 2.0 ml of standard buffer were incubated at 37 ° C. The minus enzyme incubation mixture contained the same amount of glycerol as the plus enzyme mixture. After 6 h incubation, 0.3 ml of each reaction mixture was applied directly to a column (1.6 x 80 cm) of Bio Gel A5m equilibrated with 0.1 M Tris-HCl, pH 7.4, 0.15 M NaCl, 1 mM EDTA, 0.01% NaN_3 and operated at room temperature. The column was eluted with the equilibration buffer at a flow rate of 10 ml/h.

FIGURE 5. Zonal ultracentrifugation of HDL_2 and HDL_2 incubated with hepatic lipase. The reaction mixtures were identical to those described in Fig. 4. After incubation for 6 h at 37 ° C, total HDL (d 1.063-1.210 g/ml; 60 mg protein) were added to 2 ml of the reaction mixture and the sample was immediately subjected to zonal ultracentrifugation as described in Materials and Methods. (A) Minus hepatic lipase. (B) Plus hepatic lipase. For conciseness, only the HDL profile is shown; albumin was present at d > 1.25.

hydrolyzed. After incubation with hepatic lipase, HDL_2 were reisolated by ultracentrifugation at d= 1.210 g/ml. The phospholipid to protein ratio of control HDL_2 was 0.74. The corresponding ratio in HDL_2 incubated with hepatic lipase was 0.59, indicating a loss of phospholipid relative to protein. The alteration in HDL_2 composition was also reflected in the chromatographic and ultracentrifugation properties. Chromatography of HDL_2 which were incubated with hepatic lipase showed a decrease in the size of the particle as compared to the control HDL_2; the released [^{14}C] palmitic acid and [^{14}C] lysopalmitoyl phosphatidylcholine in the enzyme-treated HDL_2 were associated with the albumin fraction (Fig. 4). Zonal ultracentrifugation of HDL_2 is shown in Fig. 5.

In this experiment, radiolabeled HDL_2 were incubated with hepatic lipase and then total HDL (d 1.063-1.210 g/ml) were added; the mixture was subjected immediately to ultracentrifugation. HDL_2 which were incubated with albumin in the absence of the enzyme were isolated at a peak density of 1.136 g/ml, whereas the HDL_2 which were incubated with hepatic lipase had a peak density of 1.141 g/ml.

DISCUSSION

The results of the present study are of interest as they relate to two recent reports. Musliner et al. (18) showed that total human HDL triglycerides were hydrolyzed by human hepatic lipase in vitro. Van Tol et al. (19) have shown that rat heparin-releasable hepatic lipase hydrolyzed HDL_2 phospholipids; no attempt was made to determine the hydrolysis of HDL_2 triglycerides or to determine the lipoprotein specificity of the enzyme. The present report using human lipoproteins shows that hepatic lipase hydrolyzes not only HDL_2 phospholipids but also HDL_2 triglycerides. In addition, HDL_2 was the preferred substrate for the enzyme; HDL_3 phospholipids were hydrolyzed to a lesser extent. Lipoprotein lipase had negligible activity on HDL_2 triglycerides. At comparable enzyme concentrations, lipoprotein lipase hydrolyzed VLDL triglycerides more readily than hepatic lipase, whereas with HDL_2 triglycerides, hepatic lipase was more active. These in vitro data with human lipoproteins are consistent with clinical correlations observed in man (7,20). Elevated heparin-releasable hepatic lipase activity is associated with decreased concentrations of plasma HDL_2 phospholipids and cholesterol; low activity is associated with high concentrations of HDL_2 lipids (7). In contrast, lipoprotein lipase activity in postheparin plasma is directly correlated to HDL_2 lipids and indirectly to VLDL-triglycerides (20).

The finding that hepatic lipase hydrolyzes both HDL_2 phospholipids and triglycerides suggests that it may play a role in the interconversion of HDL subfractions. In this regard, Jansen and Hulsmann (21) have suggested that the depletion of phospholipids from HDL_2 by the action of liver lipase induces a transfer of unesterified cholesterol to tissues, the possible end result being the transformation of HDL_2 into HDL_3. HDL_3 could then serve as an acceptor for phospholipids and apoproteins produced during the hydrolysis of triglyceride-rich lipoproteins by lipoprotein lipase, as has been demon-

strated in vitro (22) and in vivo after heparin injection (23). The data in Fig. 5 suggest that hepatic lipase alone does not produce HDL_3 from HDL_2 in vitro. Other components within the plasma compartment may be required for this conversion. For example, specific lipid transfer proteins may be required for the removal of core lipid components within HDL_2 during catabolism by hepatic lipase. Experiments to test the possible role of lipid transfer proteins in the interconversion of HDL are currently in progress.

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