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ACTIVATION OF HUMAN POST HEPARIN LIPOPROTEIN LIPASE BY APOLIPOPROTEIN H (β_2-GLYCOPROTEIN I)

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SUMMARY: Lipoprotein lipase (LPL) is the major enzyme involved in triglyceride hydrolysis of lymph chylomicrons and plasma very low density lipoproteins. LPL can be isolated from human post heparin plasma by heparin-Sepharose 4B affinity chromatography. In the present study the effects of apolipoproteins (apo) C-II, C-III, and H on the enzymic activity of LPL were investigated. ApoH is a recently described protein (β_2-glycoprotein I) constituent of triglyceride rich lipoproteins in human lymph and plasma. Human LPL was activated by apoC-II, and the apoC-II activation of LPL was inhibited by apoC-III. ApoH increased the enzymic activity of LPL in the presence of apoC-II by 45 ± 17 percent. ApoC-III decreased the apoH + apoC-II enhanced activity of LPL by 77 percent. These results provide evidence for the concept that the enzymic activity of LPL in triglyceride metabolism is modulated by apoH. The relative proportion of apoH, apoC-II, and apoC-III in triglyceride rich lipoprotein particles may determine the ultimate rate of LPL catalyzed triglyceride hydrolysis.

Human post heparin plasma has been shown to contain two major lipolytic enzymes, hepatic lipase and lipoprotein lipase[1] (1-3). LPL is an extracellular enzyme attached to the capillary endothelium and is responsible for hydrolysis of triglyceride rich plasma lipoproteins (4). Triglyceride hydrolysis results in a change in hydrated density of triglyceride rich lipoproteins resulting in conversion of liver VLDL to LDL, and the catabolism of intestinal chylomicrons to chylomicron remnants. The specific function of hepatic lipase in lipoprotein metabolism is as yet unknown.

Previous studies have shown that two major plasma apolipoproteins, apoC-II and apoC-III, modulate the enzymic activity of LPL. ApoC-II increases LPL activity, and apoC-III reduces the apoC-II activation of human and rat LPL as well as bovine milk LPL (5-10).

1. Abbreviations: lipoprotein lipase, LPL; very low density lipoproteins, VLDL; low density lipoproteins, LDL; high density lipoproteins, HDL; apolipoproteins, apo; bovine serum albumin, BSA.

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Recently β_2-glycoprotein-I has been shown to be a protein constituent of human plasma chylomicrons and VLDL, and to be present in the 1.23 g per ml infranate (11). β_2-glycoprotein-I was also reported to have a high affinity for Intralipid (12). The results in the present report indicate that β_2-glycoprotein-I increases the enzymic activity of LPL. Since β_2-glycoprotein-I is associated with plasma lipoproteins, has a high affinity for lipid, and enhances the catalytic activity of LPL, we have designated β_2-glycoprotein-I for consistency as well as convenience as apoH in accord with the alphabetized nomenclature of plasma apolipoproteins (13).

MATERIALS AND METHODS

Enzyme Source. Human post heparin plasma was obtained from normal fasting subjects following intravenous injection of sodium heparin (75 U/kg body weight, beef lung heparin, Upjohn Co.). Blood was collected 15 min after injection, mixed with 0.25 M sodium citrate (0.05 ml/ml blood, J. T. Baker Chemical Co.), and centrifuged at 2000 rpm for 30 min in a Sorvall RC-3 centrifuge (Dupont Instruments) at 4°C. The plasma was aspirated and stored at -70°C until used.

LPL was separated from post heparin plasma by heparin-Sepharose affinity chromatography. Heparin-Sepharose 4B (Pharmacia Fine Chemicals) was prepared as described by Iverius (14), using intestinal mucosa heparin (Hynson, Westcott, and Dunning, Inc.). Heparin-Sepharose 4B columns (Isolab, QS-6E, 0.6 X 1.75 cm) were prepared and equilibrated in 5mM barbital buffer (pH 7.4) containing 0.4 M NaCl (Buffer A). Two ml of post heparin plasma was thawed just prior to use and diluted with 2 ml of Buffer A. The plasma-Buffer A solution was applied to the column, and the column was washed with 8 ml of Buffer A. Hepatic lipase was eluted from the column with 5 ml of 5 mM barbital buffer (pH 7.4) containing 0.7 M NaCl. LPL was then eluted (0.5 ml fractions) from the column with 1 ml of 5 mM barbital buffer (pH 7.4) containing 1.5 M NaCl. Following elution LPL (0.2 ml) was stabilized by the addition of 0.05 ml of 5 mM barbital buffer (pH 7.4), 1.5 M NaCl containing 10% (weight/volume) BSA (Miles Laboratory, Inc., fraction V, fatty acid-free).

Substrate. [9, 10 (*n*)-^{3}H] glycerol trioleate ([^{3}H] triolein, 397 mCi/mmole, Amersham Corp.) in toluene and unlabeled triolein (Applied Science Lab., Inc.) were repurified by column chromatography using silicic acid (Mallinckrodt, 100 mesh) and Florisil (Fisher, 60-100 mesh) (15). Petroleum ether (Burdick and Jackson Lab., Inc.) and anhydrous ethyl ether (Mallinckrodt) were used for column chromatography. Anhydrous emulsions of [^{3}H] triolein in glycerol (J. T. Baker Chemical Co.) were prepared as described by Nilsson-Ehle and Schotz (15) using lecithin from egg yolk (Sigma) as stabilizer. Assay substrate (7.2 mM [^{3}H] triolein, 9.9 x 10^3 dpm/nmol triolein, 2% BSA, 0.13 M Tris Buffer pH 8.6) were prepared daily by dilution of anhydrous emulsions with 0.15 M Tris Buffer (pH 8.6) containing 2.3% BSA.

Assay Systems. Assays of lipoprotein lipase were performed with 3.6 mM [^{3}H] triolein, 90 mM Tris Buffer (pH 8.6), 2% BSA, 30 mM NaCl, in a final volume of 0.1 ml. The assay mixture contained enzyme and purified apolipoprotein concentrations indicated in each experiment.

All incubations were initiated by addition of assay substrate (50 µl) and were carried out at 37°C in a shaking water bath. Reactions were terminated after 30 minutes by addition of 0.75 ml of modified Dole's extraction mixture (2% 1N H_2SO_4, 78% isopropyl alcohol, 20% n-hexane) (16). Reaction products were separated by the addition of 0.35 ml of H_2O and 0.45 ml of n-hexane. 0.5 ml of the upper phase was mixed with 0.75 ml of 0.05 M NaOH-50% ethanol solution, and fatty acids were recovered in the NaOH-ethanol solution. Radioactivity in the NaOH-ethanol extracts was quantitated in a Scintillation Spectrometer (Searle Analytic, Inc.) using Aquasol (New England Nuclear) as scintillator.

One mU of enzyme activity was defined as the release of 1 nanomole of oleic acid per minute at 37°C. Enzyme activity was expressed per ml of enzyme preparation. The mean value (±SD) of LPL specific activity was 6.96 ± 1.13 mU/µg protein at an apoC-II concentration of 2.5 µg/ml in 90 mM Tris, 2% BSA, 30 mM NaCl, pH 8.6 buffer.

Protein concentration of enzyme was determined by fluorometric assay (fluorescamine, Roche) using a Perkin-Elmer MPF-4 fluorescence spectrometer (17). BSA (Pentex) was used as standard.

Purification of Apolipoproteins. ApoC-II and apoC-III_2 were isolated from human plasma VLDL and HDL by DEAE (Whatman) chromatography as previously reported (18). ApoH was isolated from human thoracic duct lymph (density <1.019 g/ml) and from plasma lipoproteins of density <1.019 g/ml from a patient with type V hyperlipoproteinemia by heparin affinity chromatography followed by fractionation on Sephacryl S-300 (Pharmacia) as previously described (19, 20). All proteins migrated as a single electrophoretic band on sodium dodecyl sulfate polyacrylamide gel electrophoresis and were immunochemically homogeneous when assayed against antisera to all other known apolipoproteins.

RESULTS

Previous reports have indicated that apoC-II and apoC-III modulate the enzymic activity of LPL (5-10). In our studies, LPL, purified from post heparin plasma by heparin affinity chromatography, was maximally activated 21 ± 4 fold (n=10) by 2.5-10 µg/ml of apoC-II. Concentrations of apoC-II greater than 10 µg/ml were associated with less activation of enzymic activity.

ApoH, purified from human plasma lipoproteins or thoracic duct lymph, also stimulated the enzymic activity of LPL. In experiments (n=5) using LPL isolated from normal subjects (n=2) apoH increased LPL enzymic activity (assayed in the presence of 2.5 µg/ml apoC-II) by 45 ± 17% above control values (Fig. 1). ApoH also caused a slight activation of LPL activity in the absence of apoC-II (Fig. 1).

ApoC-III_2 inhibited the enhanced enzymic activity of LPL produced by apoC-II (2.5 µg/ml), and apoH (20 µg/ml) + apoC-II (2.5 µg/ml). At maximum

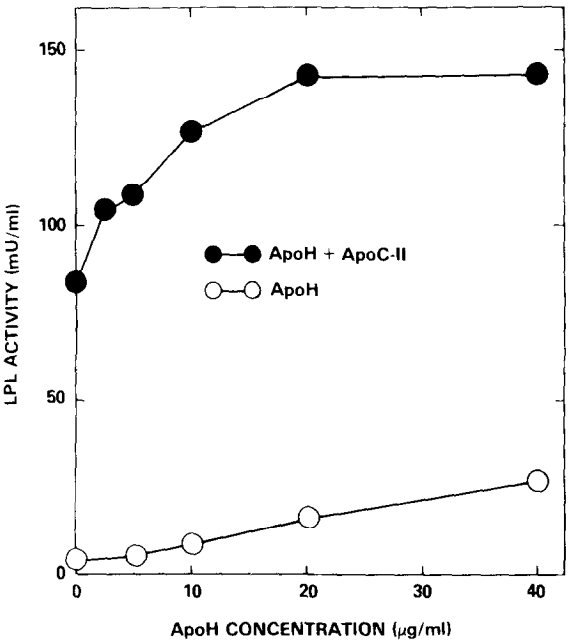

Fig. 1. The effect of apoH on the enzymic activity of lipoprotein lipase in the presence and absence of apoC-II (2.5 μg/ml). Experiments were carried out in buffer containing 90 mM Tris, 2% BSA, 30 mM NaCl, pH 8.6 (enzyme concentration, 0.21 μg per ml of assay solution). Each point on the graph represents the mean of two determinations.

concentrations of apoC-III_2 (40 μg/ml), an 86% inhibition of apoC-II, and a 77% reduction of apoH + apoC-II stimulated activity of LPL were observed.

DISCUSSION

Several reports have suggested that the weight ratio of apolipoproteins C-II and C-III are important determinants of the enzymic activity of LPL, and the catabolism of triglyceride rich plasma lipoproteins (7-10, 21, 22). In plasma VLDL isolated from patients with Type V hyperlipoproteinemia this ratio has been reported to be lower resulting in a relative deficiency of apoC-II and an impairment in triglyceride metabolism (21, 22). ApoH is a protein recently recognized to be associated with lymph and plasma lipoproteins (11), and to have a high affinity for artificial lipid complexes (12). In the present studies apoH was shown to enhance the enzymic activity of LPL in the presence of apoC-II. ApoC-III inhibited the activation of LPL by apoH + apoC-II. The further increase in enzymic activity of LPL in the presence of

apoC-II by apoH indicates that this apolipoprotein may play an important role in regulating LPL activity. The results from these studies provide evidence for the concept that the enzymic activity of LPL in triglyceride metabolism is modulated by several apolipoproteins including apoH, apoC-II, and apoC-III. These findings may have important implications in elucidating the molecular mechanism involved in triglyceride metabolism in normal and hypertriglyceridemic subjects.

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