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MODULATION, BY APOLIPOPROTEIN E, OF LIPOPROTEIN LIPASE ACTIVITY

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SUMMARY

The effects of apolipoprotein E and its antibody on the activity of lipoprotein lipase were studied. Apolipoprotein E was first subjected to heparin-Sepharose 4B affinity chromatography, and further purified by preparative isoelectric focusing. Antibody specific to this apolipoprotein was raised in a rabbit. Lipoprotein lipase was purified from human postheparin plasma using heparin-Sepharose 4B affinity chromatography. Apolipoprotein E significantly activated lipoprotein lipase, while activity of this enzyme was inhibited by apolipoprotein E antibody. These findings support a role for apolipoprotein E in lipoprotein lipase-mediated triglyceride catabolism.

INTRODUCTION

Triglyceride-rich lipoproteins are hydrolyzed by lipoprotein lipase, located at the surface of the capillary endothelium. Intravenous injection of heparin releases into the blood circulation two triglyceride lipases, i.e. lipoprotein lipase (LPL) and hepatic triglyceride lipase (1). Several apolipoproteins have been implicated in the regulation of the activity of LPL. Since apolipoprotein CII has been shown to be a specific activator for LPL, it appears to be the most important (2). However, apolipoprotein E (apoE) has been shown to have a high affinity for heparin similar to that possesed by LPL (3), suggesting that it too may play a role in regulating LPL. Nevertheless, reports concerning the effects of apoE on LPL have been contradictory (4, 5, 6). The aim of the studies reported here was to examine the possible role of apoE in the regulation of LPL. To accomplish this, we examined the effects of pure apoE and antibody specifically prepared against this apolipoprotein on LPL purified from postheparin plasma.

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MATERIALS AND METHODS

Purification of apoE and production of its antibody Very low density lipoproteins (VLDL) were separated from the plasma of normolipidemic subjects by ultracentrifugation, using a Beckman SW-27 rotor (7). VLDL were dialyzed against 200 vol of distilled water containing 0.01% EDTA, pH 7.4. After lyophilization, these lipoproteins were delipidated 5 times with chloroform-methanol (2:1, V/V). The apo VLDL (3-4 mg/ml) dissolved in 2 mM sodium phosphate buffer containing 0.05 M NaCl and 6 M urea (pH 7.4) were applied to a 1 x 6.5 cm column of heparin-Sepharose 4B equilibrated with the same buffer (3). The column was washed with 100 ml of the buffer in 0.05 M NaCl, then eluted with 10 ml of the buffer at an ionic strength of 0.5 M NaCl. Apolipoproteins in this eluate were concentrated and subjected to preparative isoelectric focusing in a gel containing 6% polyacrylamide, 8 M urea and 2.0% amphorine (pH 5-8, LKB), run at 4°C for 18 hr at a constant 400 volt. Gel fraction corresponding to apoE was homogenized and stirred overnight at 4°C, and the eluted proteins were re-chromatographed on heparin-Sepharose 4B to remove the amphorines. The purity of the apoE thus obtained was evaluated by sodium dodecyl sulfate (SDS) 11% polyacrylamide gel electrophoresis (8).

ApoE (500 µg) was injected intradermally into a laboratory rabbit every two weeks for 5 times. Serum was ultracentrifuged at a density of 1.21 g/ml to remove lipoproteins. The γ-globulin fraction was prepared by ammonium sulfate precipitation of serum at 50% saturation, followed by a second precipitation at 33% saturation. The precipitates were dissolved in saline and dialyzed against 15 mM Tris-phosphate buffer (pH 8.3). The dialyzate was then subjected to DEAE-cellulose chromatography, and the γ-globulin fraction was dialyzed against 0.02 M borate buffer in 0.15 M NaCl (pH 8.0) (9).

Purification of human LPL Ten minutes after intravenous injection of heparin (100 U/Kg) into normolipidemic subjects, blood was collected. Plasma was separated in a refrigerated centrifuge and applied to a 1 x 5 cm column of heparin-Sepharose 4B equilibrated with a buffer of 5 mM veronal-HCl containing 0.4 M NaCl (pH 7.4). The column was washed with 0.4 M NaCl buffer, then a stepwise elution was carried out at 4°C using 0.7 M NaCl buffer followed by 1.5 M NaCl buffer (10, 11).

Assay of LPL activity The substrate was a mixture of 2 µCi of [^{14}C] triolein (Amersham/Seale Corp.), 0.133 g of unlabelled triolein (Sigma Chem.), 0.9 ml of 1% Triton X-100, 0.9 ml of 4% bovine serum albumin solution adjusted to pH 8.6 and 10.2 ml of 0.2 M Tris-HCl buffer (pH 8.6). The mixture was sonicated on ice for 3 min with a Tomy-UP sonifier (Tomy, Seiko, Tokyo). To 0.4 ml of this substrate, was added 0.05 ml of pooled human plasma which had been preincubated for 30 min at 37°C with variable amounts of apoE diluted with 0.02 M sodium phosphate buffer (pH 8.0), or variable amounts of anti-apoE γ-globulin solution diluted with normal rabbit γ-globulin solution prepared in the same way as the anti-apoE γ-globulin. Volume was adjusted to 0.9 ml by adding 4% bovine serum albumin/0.2 M Tris-HCl buffer (pH 7.4). Assay was started with the addition of 0.1 ml of purified LPL solution and incubation was carried out for 30 min at 37°C. Radioactivity of the free fatty acids released during incubation was measured and LPL activity was determined (10, 11).

RESULTS

Purification of apoE and production of its antibody The identity of the apolipoprotein partially purified by heparin-Sepharose 4B affinity chromatography and further purified by preparative isoelectric focusing, was shown to be pure apoE by SDS polyacrylamide gel electrophoresis (Fig. 1). The molecular

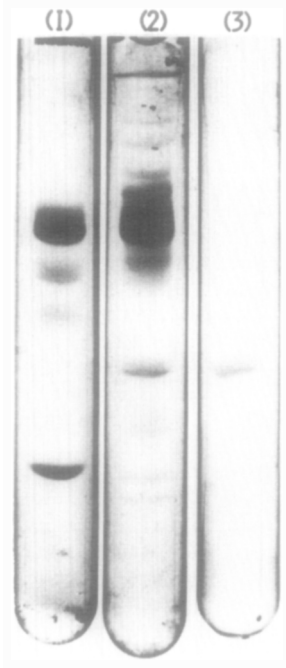


Fig. 1 SDS polyacrylamide gel electrophoresis of apolipoproteins in (1) the breakthrough fraction of the heparin-Sepharose affinity column, (2) the fraction eluted from the column with 0.5 M NaCl and (3) apoE purified by preparative isoelectric focusing.

weight of this protein was about 33,500-34,500 daltons on SDS polyacrylamide gel electrophoresis and the pI of this protein ranged from 5.8 to 6.3. These characteristics coincide with those previously described for apoE (12, 13). ApoE antibody had no cross reactivity with any other apolipoproteins or albumin both by double immunodiffusion (Fig. 2) and by immunoelectrophoresis.

Purification of LPL postheparin plasma two lipases, i.e. hepatic triglyceride lipase and LPL, were separated by heparin-Sepharose 4B affinity chromatography with 0.7 M NaCl and 1.5 M NaCl, respectively (10, 11). LPL was purified 1900-fold and revealed a single major band on SDS polyacrylamide gel electrophoresis. This LPL had no cross reactivity with antiserum specifically prepared against hepatic triglyceride lipase (11).

Effects of apoE and its antibody on LPL activity LPL was activated by the addition of pooled human plasma, with maximum activation attained after

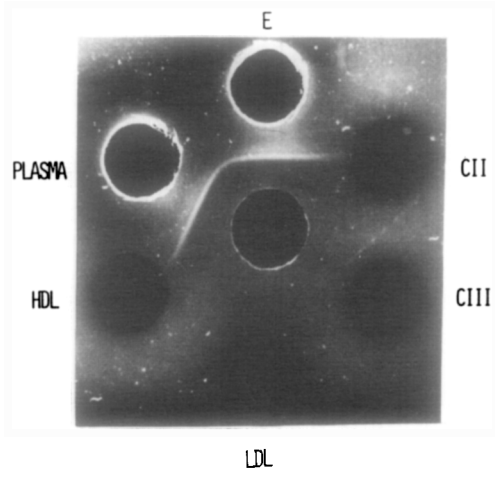


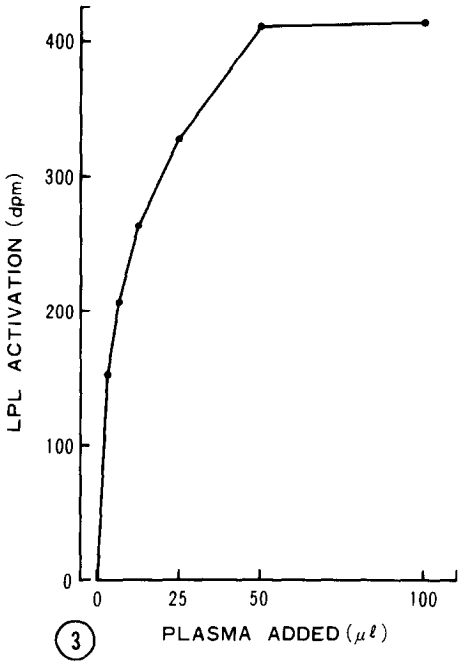
Fig. 2 Double immunodiffusion of anti-apoE γ-globulin (center well) to apoE, apoCII, apoCIII, LDL, HDL and plasma.

addition of 0.05 ml of plasma (Fig. 3). The addition of 5 μg of apoE to the LPL assay medium in which 0.05 ml of plasma was already present, resulted in a further increase in LPL activity ($p<0.02$). LPL activity was doubled by the addition of 50 μg of apoE ($p<0.001$). On the other hand, when antibody was added to the assay medium, LPL activity was significantly decreased ($p<0.001$) (Fig. 4). A dose-dependent relationship was found between the amount of antibody added and the decrease in LPL activity.

DISCUSSION

In humans, chylomicrons and VLDL are rich in apoE, while other lipoprotein fractions contain very little of it. The observation that upon entering the blood circulation, triglyceride-rich lipoproteins receive apoE from high density lipoproteins suggests that this apolipoprotein may participate in the catabolism of these lipoproteins (14). There is also suggestion that apoE has a high affinity for heparin (3). Since LPL has also been known to have an affinity for heparin, high affinity of apoE for heparin suggests the possibility for an interaction between this apolipoprotein and LPL

In an attempt to define this possibile association, we conducted this study. Previous workers have reported conflicting observations on the effect



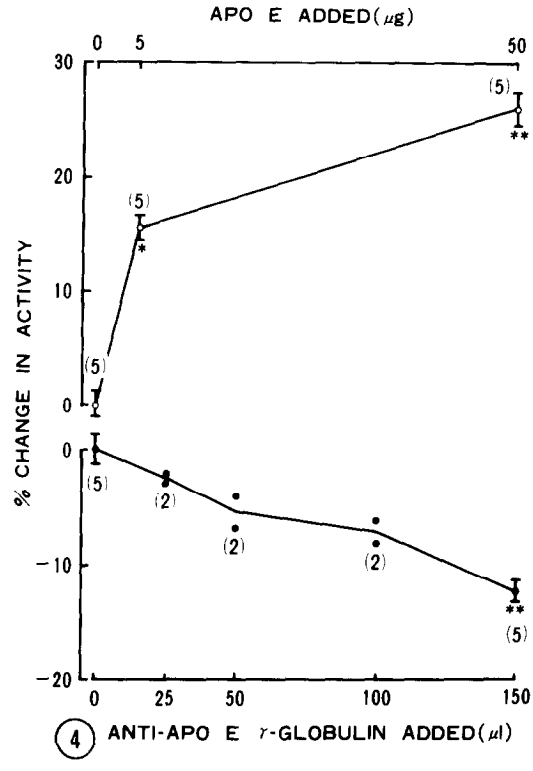


Fig. 3 The effect of adding varying amounts of plasma on the activity of LPL purified by heparin-Sepharose affinity chromatography. Activity of LPL is expressed as dpm.

Fig. 4 The effects of apoE (upper) and its antibody (lower) on the activity of purified LPL in a system which had been maximally activated by optimal amount of normal pooled plasma. In parentheses, are indicated the number of determinations. * $p<0.02$, ** $p<0.001$ (compared to the enzyme activities measured in a system free from apoE or its antibody)

of apoE upon LPL; apoE activated (4), suppressed (5) or had no effect on LPL (6). Since these contradictory results may be due to differences in the conditions of the enzyme assay, as well as in the purity and source of the apolipoprotein and the enzyme, in the present study, the effects of pure apoE and its antibody on LPL activity were explored in a assay system in which purified LPL had been maximally activated by plasma. The results indicated that pure apoE activated purified LPL. Furthermore, the selective removal of apoE by a specific antibody significantly decreased LPL activty. From these observations as well as the previous finding that apoE is transferred from high density lipoproteins to triglyceride-rich lipoproteins when they enter

the circulation, we conclude that apoE, through activation of LPL, may be an important factor in LPL-mediated triglyceride catabolism.

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