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Inhibition of lipoprotein lipase by the receptor-binding domain of apolipoprotein E

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A synthetic peptide (residues 139–153) corresponding to the receptor-binding domain of apolipoprotein E (ApoE) was tested for lipoprotein lipase (LPL) inhibitory properties. In systems using both natural and synthetic substrates, inhibition of LPL was observed. Using the synthetic substrate, 50% inhibition was observed at 50 μM while high concentrations completely inhibited LPL activity. These studies suggest an additional functional role for the receptor-binding domain of ApoE – modulation of LPL activity.

Lipoprotein lipase; Apolipoprotein E; Receptor; Enzyme inhibition; Synthetic peptide

1. INTRODUCTION

Triacylglycerol (TG)-rich lipoproteins during their lipolysis catalyzed by lipoprotein lipase (LPL) become smaller and are either removed by high-affinity receptors (ApoE and/or ApoB/E) or converted to plasma LDL [1,2]. The catalytic rate of LPL in this process is governed both by passive modulation of TG uptake related to changes in plasma TG concentrations and active regulation of LPL activity, e.g. apolipoproteins [3]. One apolipoprotein, ApoE, has been previously reported [4–7] to have an inhibitory effect on LPL catalytic activity. Thus, ApoE may play a dual signaling role in the clearance of plasma TG-rich lipoproteins: one regulating the rate of lipolysis, and the other, uptake by high-affinity receptors [1,2]. Recently, the receptor-binding domain (RBD) of human ApoE has been localized and characterized by Mahley et al. [8]. Because of our interest in studying ApoE receptor interactions by competition experiments, we have prepared the synthetic peptide representing the RBD of ApoE (residues 139–153). Since ApoE inhibits LPL activity [4–7] we tested RBD for its possible inhibitory potential in an LPL assay system. The subject of this paper is to report that the synthetic peptide corresponding to the RBD of ApoE represents an important domain in the inhibition of LPL.

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Abbreviations: LPL, lipoprotein lipase; ApoE, apolipoprotein E; RBD, receptor-binding domain; TG, triacylglycerol; VLDL, very low density lipoproteins

2. MATERIALS AND METHODS

2.1. *Peptide synthesis*

The RBD synthetic peptide (residues 139–153 of ApoE) was synthesized on an Applied Biosystem synthesizer (model 430A) at the Molecular Biology Resource Facility, Oklahoma University Health Sciences Center. Peptide synthesis was performed using the solid-state methodology as first described by Merrifield [9]. Briefly, the peptide was synthesized on a solid support by stepwise addition of N-protected amino acids. At the completion of the synthesis, the peptide was cleaved from its solid support and permanent side-chain-protecting groups by HF [10]. Synthesized peptides were purified by preparative reversed-phase chromatography. Characterization of purified peptides included a single peak by analytical reversed-phase

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high-pressure liquid chromatography and the expected amino acid analysis.

2.2. *Lipoprotein lipase*

2.2.1. Preparation of LPL and substrates

Bovine milk LPL was used in these studies. The purification of bovine milk LPL using heparin-Sepharose affinity chromatography was performed as described by Posner et al. [11].

2.2.2. Assay

The assay of lipoprotein lipase activity was performed with lecithin (dioleoylphosphatidylcholine)-emulsified glycerol tri-[9,10-^{3}H]oleate as substrate. The molar ratio of lecithin to trioleoylglycerol was 1:10. The 2-fold concentrated substrate was prepared as described by Eckel et al. [12]. The assay mixture contained 1.4 mM trioleoylglycerol, 60 mg/ml of bovine serum albumin, 3% human serum as the source of lipase activator, and an appropriate volume of 100-fold diluted enzyme (10–20 μl). The final volume of the assay mixture was adjusted to 100 μl with the addition of 50 mM NH_4OH-HCl buffer, pH 8.5. The mixture was shaken on a water bath at 37°C for 1 h. The reaction was terminated by adding 3.2 ml chloroform/heptane/methanol (5:4:5.6, v/v) and 1 ml of 0.2 N NaOH. After centrifugation, 1.2 ml of the top layer was mixed with 10 ml Instagel (Packard) and the radioactivity measured in a Packard liquid scintillation counter. One unit of activity is defined as 1 nmol fatty acid released per h at 37°C. Alternatively, when a VLDL substrate was used, the assay mixture was extracted as previously described for determination of the first-order rate constant (k_1) of TG degradation by gas-liquid chromatography [13].

Table 1

Inhibition of lipoprotein lipase (LPL) by receptor-binding domain of ApoE (residues 139–153, M_r 1859)

Substrate	ApoE RBD (μM)	Inhibition[a] (%)
Native VLDL[b]	2.4	2.8
	23.9	17.8
	113.1	32.9
Artificial substrate	433	100
	418	99
	87	68
	17	27
Artificial substrate[c]		
Acetic acid	98.2	65.3
Albumin	195.7	90.4
DMSO	233.3	97.6
DMSO	87.7	88

[a] Based on control activity

[b] Degree of inhibition based on change in first-order rate constant (k_1)

[c] Different agents used to solubilize synthetic peptide before addition to substrate

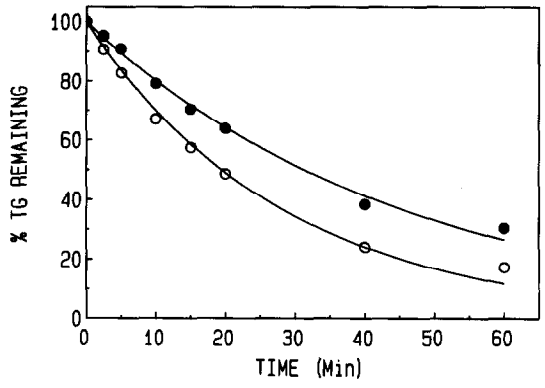

Fig.1. Effect of RBD of ApoE on degradation of VLDL. Disappearance curve of TG following degradation of VLDL by LPL in the presence of RBD of ApoE [(●) 113 μM] and its absence (○). Plots are best-fit exponential curves as described by Wang et al. [13].

2.2.3. Inhibition assay

In the inhibition assay, the synthetic peptide of ApoE was added to the assay mixture followed by 20 μl diluted bovine milk LPL. Appropriate controls containing the solvent in each case were assayed with only the peptide absent.

3. RESULTS

The synthetic RBD of ApoE was solubilized in several different buffers and tested using both native and synthetic substrate systems (table 1). The RBD of ApoE could, at higher concentrations, completely inhibit LPL activity. LPL inhibitory properties were also observed with a shorter synthetic peptide (residues 141–153) which had its N-terminal acetylated. As illustrated

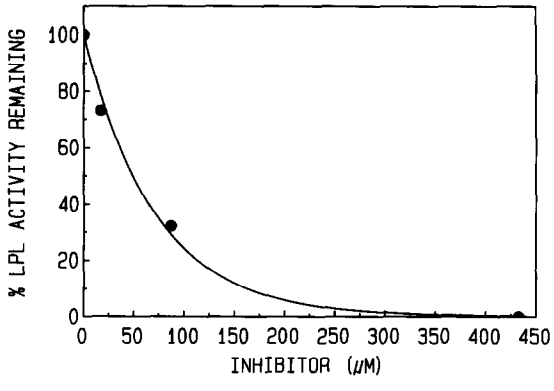

Fig.2. Effect of RBD of ApoE on LPL activity. Synthetic substrate system used and different concentrations of RBD of ApoE (residues 139–153) added.

(fig.1), the addition of RBD of ApoE to VLDL decreased (33%) the first-order rate constant (k_1) for the disappearance of VLDL-TG from 0.0348 to 0.0221 min^{-1}. In addition, a plot of the inhibition of synthetic substrate degradation using dilutions of the RBD of ApoE (fig.2) demonstrated that an estimated $K_{i,50}$ was of the order of 50 μM as estimated from the concentration of RBD of ApoE necessary to achieve 50% inhibition of TG degradation.

4. DISCUSSION

The synthetic peptide corresponding to the RBD of ApoE has striking inhibitory properties in assay systems containing either VLDL or a synthetic substrate. Differences in inhibition effect of RBD of ApoE using the native or synthetic substrate are due to compositional variations in the substrates. The estimation of a $K_{i,50}$ of the order of 50 μM with the synthetic substrate suggests that this domain may represent a physiologically important regulator of plasma TG levels. In conjunction with the role of this domain in receptor binding, such an observation indicates that the exposure of the RBD of ApoE during lipolysis of TG-rich lipoproteins could inhibit LPL and thus direct the partially degraded TG-rich lipoprotein to high-affinity receptor-mediated uptake by the liver. Such a competitive mechanism could be important in regulating plasma TG levels by both modulating LPL activity and clearance of TG-rich lipoproteins by receptor-mediated uptake.

A number of studies have reported changes in ApoE structural properties in relation to the in vitro characteristics of VLDL following perturbations including lipolysis [14,15], proteolysis [16], size characteristics of VLDL particles [17] and ApoE epitope exposure [17,18], and lipid exchange [14]. In addition, phospholipids have been shown to be necessary for ApoE binding to receptor [19]. All of these studies clearly demonstrate that the surface properties of VLDL as mediated by the conformation of ApoE could alter the in vivo fate of TG-rich lipoproteins or their remnants.

This study provides new information regarding the central role played by the RBD of ApoE in regulating the conversion and clearance of TG-rich lipoproteins mediated by LPL and points to a defined polypeptide probe for future studies on the interaction of ApoE with LPL.

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