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LIPOLYSIS OF ApoC-II DEFICIENT VERY LOW DENSITY LIPOPROTEINS: ENHANCEMENT OF LIPOPROTEIN LIPASE ACTION BY SYNTHETIC FRAGMENTS OF ApoC-II

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SUMMARY

Enzymic hydrolysis of triacylglycerol has been studied with very low density lipoproteins from an individual with a genetically determined absence of apoC-II, the activator apoprotein for lipoprotein lipase. Normal rates of ester cleavage by purified bovine milk lipoprotein lipase can be achieved *in vitro* with native apoC-II and by three shorter synthetic peptides, apoC-II(55-78), apoC-II(50-78) and apoC-II(43-78), which contain part of the carboxyl terminal third of the native apoprotein. At 0.5 μM concentration, all peptides produced a 7-fold activation. ApoC-II(43-78), but not apoC-II(50-78) or apoC-II(55-78), could bind VLDL as shown by separation of unbound ^{125}I peptides and the lipoproteins. Thus, residues 43-50 of apoC-II are part of a lipid binding region. High affinity binding of apoC-II peptides to the lipoprotein substrate is not obligatory for activation of lipoprotein lipase.

Triacylglycerol is transported in blood by chylomicrons and very low density lipoproteins (VLDL) from the intestine and liver (1,2). The initial catabolism of these triacylglycerol-rich lipoproteins occurs by ester hydrolysis at the vascular endothelium of extrahepatic tissues, catalyzed by lipoprotein lipase (EC 3.1.34). Maximal hydrolysis requires activation of the enzyme by apoprotein C-II (apoC-II), a protein constituent of the lipoprotein substrate (3-6). With synthetic fragments of apoC-II, we have shown that the sequence of apoC-II residues (55-78) (7) contains the determinants for activation of lipoprotein lipase.

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Breckenridge et al. have described a deficiency of apoC-II that leads to hypertriglyceridemia (8). Infusion into the patient normal plasma or high density lipoproteins, which contain apoC-II, decreased the plasma triacylglycerol. The aims of the present study have been to determine if native and synthetic fragments of apoC-II could promote lipoprotein lipase hydrolysis of triacylglycerol in this apoC-II deficient VLDL and to identify distinct regions of sequence involved in lipid binding and in activation of the enzyme.

MATERIALS AND METHODS

Source of ApoC-II Deficient VLDL - The subject was a 62 year old male, with maturity onset diabetes controlled by insulin. A complete description of this subject has been published (8). Chylomicrons and VLDL were isolated from fasting plasma by ultracentrifugation at plasma density for 48,000 and 792,000 g-hr, respectively. VLDL were dialyzed against 0.15 M NaCl containing 1 mM NaN_3 and sterilized by filtration through a 0.22 μ Millipore filter.

Preparation of Native and Synthetic Fragments of ApoC-II - ApoC-II was isolated (9) from the VLDL of fasting subjects with type IV or V hyperlipoproteinemia. Synthetic peptides were prepared by solid-phase techniques (7). ApoC-II(55-78), apoC-II(50-78) and apoC-II(43-78) contain amino acid residues 55 through 78, 50 through 78, and 43 through 78, respectively, of apoC-II. Purities of apoC-II and the synthetic peptides were greater than 95% by analytical isoelectric focusing (10) and by amino acid analysis.

Preparation of Lipoprotein Lipase - Lipoprotein lipase was isolated from bovine skim milk by affinity chromatography on heparin-Sepharose 4B (7,11). The isolated enzyme was homogeneous by SDS polyacrylamide gel electrophoresis with an apparent molecular weight of 55,000 and specific activity of 33 μmoles fatty acid liberated μg^{-1} protein hr^{-1} in an assay system of 0.5 μM apoC-II and trioleoylglycerol stabilized with egg lecithin.

In Vitro Lipolysis of VLDL - Hydrolysis of triglyceride in VLDL was determined by analysis of [1-^{14}C]oleic acid released from tri([1-^{14}C]oleoyl)glycerol incorporated into VLDL. To prepare radiolabeled VLDL (12), 51.8 μg of tri([1-^{14}C]oleoyl) glycerol (Amersham, 60 Ci/mole) in 70 μl of tetrahydrofuran were injected with a 100 μl Hamilton syringe into 5 ml $VLDL_o$ (15 mg triglyceride) and the mixture incubated for 16 h at 37°.

The assay system contained in 0.5 ml labeled VLDL (300 μg triacylglycerol), 10 mg fatty acid poor albumin (Sigma), 100 μmol Tris-HCl, pH 8.4, and 100 μmol NaCl. After addition of 9 ng lipoprotein lipase, apoC-II or synthetic peptides as

indicated in the figures, hydrolysis proceeded at 28° for 5, 10, 20 and 30 min in initial experiments that established a linear fatty acid release. Later experiments were conducted for 30 min. Fatty acid was separated by a liquid partition system (13) and quantified by liquid scintillation counting. For controls, reactions were stopped immediately after addition of the enzyme. Values are the mean of triplicate determinations (SD ± 6.6%).

Binding of Apoprotein to ApoC-II deficient VLDL - ApoC-II and synthetic peptides were labeled with ^{125}I (15), with a labeling efficiency of 25-30% and less than 0.5 mole ^{125}I per mole of peptide. By electrophoresis in alkaline urea, the labeled peptides had only one radioactive band that corresponded to the protein band.

RESULTS

Lipolysis of ApoC-II Deficient VLDL - Less than 2% of the triacylglycerol in apoC-II deficient VLDL was hydrolyzed in 30 min by lipoprotein lipase. When the same amount of apoC-II found in normal VLDL, 0.5 μM, was present in the reaction mixture, 9% of the triacylglycerol in apoC-II deficient VLDL was released, whereas hydrolysis of 13% of the triacylglycerol mass of normal VLDL was observed.

Since a synthetic peptide, apoC-II(55-78), gave full activation of lipoprotein lipase with a gum arabic stabilized triacylglycerol emulsion (7), this peptide and other homologs were tested with a native lipoprotein substrate, apoC-II deficient VLDL. With 0.5 μM apoC-II(55-78), approximately 8% hydrolysis was achieved in 30 min; similar results were obtained with apoC-II(50-78) and apoC-II(43-78). When increasing concentrations of activators were tested, the amount of apoC-II and synthetic peptide necessary for maximal hydrolysis was identical (Fig. 1) and gave about 7.5 fold rate enhancement. Thus, the ratio of apoC-II to triglyceride required for maximal hydrolysis is quite similar to that of normal VLDL (10). No differences in the pattern of fatty acid release were found for apoC-II, apoC-II(43-78), apoC-II(50-78) and apoC-II(55-78) (data not shown).

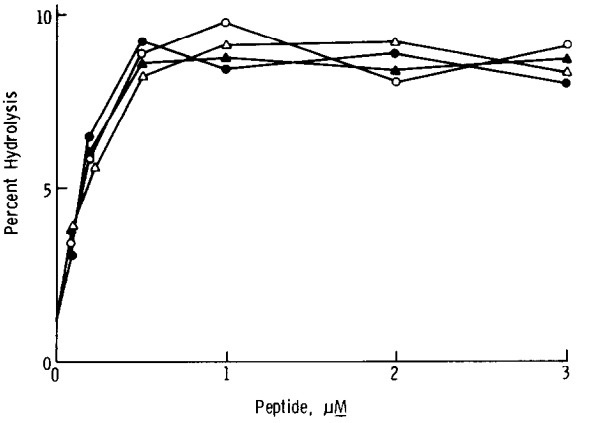

FIGURE 1 Effect of increasing concentrations of native and synthetic fragments of apoC-II on lipoprotein lipase hydrolysis of apoC-II deficient VLDL. The reaction mixture, incubated for 30 min at 27°, contained 0.5 ml, 100 μmol Tris, pH 8.4, 300 μg of VLDL triglyceride containing tri-([1-^{14}C]-oleyl)-glycerol, 10 μg bovine serum albumin and the indicated amount of peptide. ApoC-II (o-o); apoC-II(55-78) (●-●); apoC-II(50-78) (Δ-Δ); apoC-II(43-78) (▲-▲).

Binding of ApoC-II and Synthetic Peptides to ApoC-II deficient VLDL - There was little binding of apoC-II(55-78) and apoC-II(50-78) to apoC-II deficient VLDL; only 1-2% of the radioactivity was recovered with the lipoprotein (Fig. 2). By contrast, binding of apoC-II(43-78) to apoC-II deficient VLDL was about 25 times greater. Under these experimental conditions, about one-fourth of the ^{125}I-peptide was associated with the lipoprotein. For comparison, at the same concentration, greater than 90% of the apoC-II was bound to apoC-II deficient VLDL.

DISCUSSION

The severe hypertriglyceridemia in this patient has been ascribed to the absence of apoC-II (8), based on assays of isolated lipoprotein fractions. The apoC-II deficient VLDL provided a natural substrate for comparison of the abilities of apoC-II and synthetic peptide fragments of apoC-II to activate lipoprotein lipase.

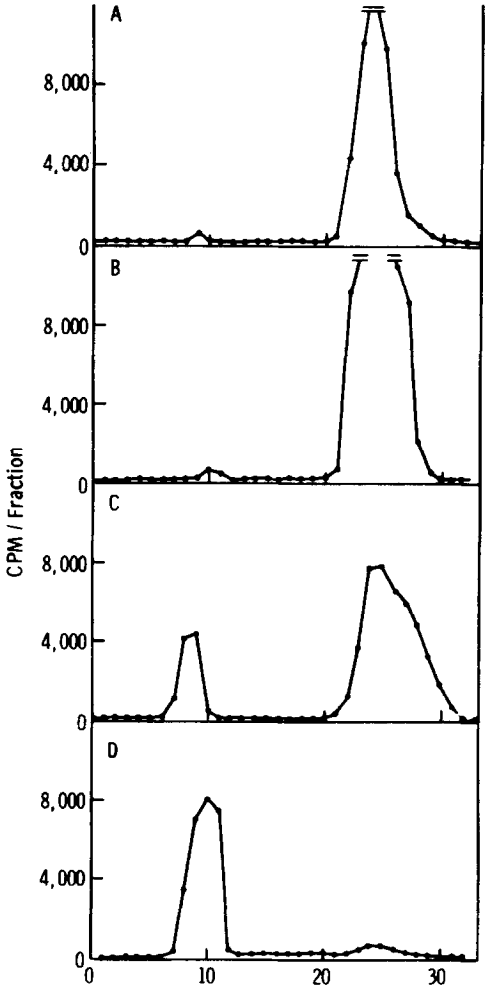

FIGURE 2 Binding of ^{125}I apoC-II and ^{125}I synthetic peptides to apoC-II deficient VLDL. After incubation of apoC-II deficient VLDL (0.3 mM triglyceride) with 0.2 μM ^{125}I peptide at 27° for 30 min, lipoproteins and unbound apoprotein were separated on Sephadex G-25, 2 x 30 cm, in 100 mM Tris-HCl, pH 7.4, containing 0.1 mM EDTA. Aliquots of 3.5 ml fractions were counted. VLDL appeared in fractions 7-11; unbound ^{125}I-peptides in fractions 21-30. The percent of binding was calculated as the proportion of the total radioactivity that eluted with the VLDL. A, apoC-II(55-78); B, apoC-II(50-78); C, apoC-II(43-78); D, apoC-II.

ApoC-II promotes triacylglycerol hydrolysis to an extent comparable to that obtained for normal VLDL and lipoprotein lipase *in vitro*. The ability of the much shorter synthetic peptides to enhance the activity of lipoprotein lipase was

very similar to that of apoC-II over a wide range of concentrations. The sequence region in apoC-II(55-78) apparently contains all the determinants required for complete activation of lipoprotein lipase. Since the synthetic peptides differed significantly from each other and from apoC-II in their abilities to bind to the apoC-II deficient VLDL, high affinity binding of the apoprotein to the lipoprotein surface is not required for hydrolysis of triglyceride by lipoprotein lipase. The formation of easily dissociated complexes of the two shorter apoC-II fragments, lipoprotein lipase and apoC-II deficient VLDL seems to be a reasonable possibility, although they are not detected by this experimental technique. Residues 43 through 50 are necessary for binding to the lipoprotein, even though this region of the peptide makes no apparent contribution to the enhancement of lipoprotein lipase activity. These results agree with those obtained with dimyristoylphosphatidylcholine, apoC-II (17) and the synthetic fragments of apoC-II (18). From our results, there appear to be other sequence regions between the amino terminal and residue 43 that contribute to lipid binding.

In conclusion, we speculate that plasma clearance of the apoC-II deficient VLDL would be normalized by apoC-II or by peptides with the minimum sequences necessary for binding to triacylglycerol-rich lipoproteins and for lipoprotein lipase activation. Synthetic fragments of apoC-II may ultimately prove to be useful therapeutic replacement agents in this deficiency disease.

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