INHIBITION OF LIPOPROTEIN LIPASE BY AN APOPROTEIN OF HUMAN VERY LOW DENSITY LIPOPROTEIN

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SUMMARY

The effects of two apolipoproteins isolated from human very low density lipoproteins (apoLp-Glu and apoLp-Ala) on lipoprotein lipase (LPL) activity have been studied. ApoLp-Glu markedly stimulated LPL as reported by others. ApoLp-Ala isolated by techniques previously described also activated LPL at low levels. However, with further purification by hydroxylapatite chromatography all activation by apoLp-Ala was eliminated with the removal of a small contaminant immunochemically identical to apoLp-Glu. ApoLp-Ala consistently inhibits LPL when present at levels above 2% of the substrate (w/w). This inhibition was not overcome by addition of phospholipid, apoLp-Glu, or more enzyme.

INTRODUCTION

Lipoprotein lipase (LPL) is an enzyme or group of enzymes which hydrolyze triglycerides only in the presence of lipoproteins or certain apolipoproteins (1,2). Activation by apolipoproteins has been demonstrated with lipoprotein lipase from rat heart (1), rat (3) and chicken (2) adipose tissue, bovine milk (4) and human plasma (5). Two specific apoproteins, apoLp-Glu and apoLp-Ala (as designated by their carboxyl terminal amino acids), have been reported to activate lipoprotein lipase (3,4). These apoproteins are present in human very low density lipoprotein (VLDL)(6,7) and as minor components in high density lipoprotein (HDL)(5,8). The present communication reports our studies on the effect of apoLp-Glu and apoLp-Ala on lipoprotein lipase activity from cow's milk.

MATERIALS AND METHODS

Lipoprotein Lipase Preparations: Skim milk from cows was prepared as outlined by

Bier and Havel (8) and dialyzed overnight versus 0.1 M sodium chloride, 0.04% EDTA, pH 7.0. The lyophilized powder (stored at -20°) was resuspended in distilled water (10 mg protein per ml as determined by the Biuret method)(10) just prior to assay. Post-heparin lipolytic activity (PHLA) was measured in plasma of normal volunteers. The blood samples were drawn 10 min after the intravenous injection of 1000 units of heparin.

Apoproteins: VLDL from the plasma of two normal subjects and four patients with Type V hyperlipoproteinemia were prepared in the ultracentrifuge, delipidated, and the proteins fractionated on Sephadex G-150 and DEAE cellulose as previously reported (6). ApoLp-Ala was further purified on columns of hydroxylapatite prepared by the method of Levin (11). Protein concentrations were determined by the method of Lowry et al. (12), using bovine serum albumin as standard. These values for apoLp-Ala protein were corrected by multiplying by 0.85 as previously reported (6). The apoproteins were dissolved in 0.05 M Tris-Cl pH 8.4 for addition to the assay mixture.

Antiserum Production: Antisera to apoLp-Glu and apoLp-Ala were prepared in guinea pigs by the subcutaneous injection of 0.25 mg of protein in 0.5 ml of complete Freund's adjuvant. After 3 weeks, a series of weekly injections of 0.25 mg of apoprotein were given until adequate reactivity by the Ouchterlony technique was obtained.

Phospholipids: Lyophilized VLDL from Type V hyperlipoproteinemic subjects was extracted initially with heptane to remove most of the neutral lipids (6). Three subsequent extractions with ethanol-ether (1:1) were combined and the phospholipids isolated on columns of silicic acid (13). Thin layer chromatography indicated less than 0.5% contamination with neutral lipids and the major phospholipids were phosphatidyl choline, sphingomyelin, lysophosphatidyl choline, phosphatidyl ethanolamine and lysophosphatidyl ethanolamine (14). Phosphorous, as determined by the method of Bartlett (15), was multiplied by 25 to obtain phospholipid concentrations. Aqueous suspensions of 1 or 2 mg/ml of phospholipid were prepared by sonic irradiation (Branson sonifier) in 0.05 M Tris-Cl, pH 8.4.

Assay of Lipoprotein Lipase Activity: ^{14}C-triolein (ICN Chemical and Radioisotope

Division) was diluted with unlabelled triolein (Sigma) to a specific activity of 0.088 μC/μmole. This triolein was emulsified in 5% gum arabic (sonic irradiation) and mixed with an equal volume of bovine serum albumin (Armour Fraction V) in a 0.2 M Tris-Cl, 0.6 M NaCl buffer of pH 8.4. This was used as "substrate". The assays were performed in 12 ml glass-stoppered centrifuge tubes containing, in a final volume of 0.5 ml: 0.1 ml "substrate" (0.76 μmoles triolein), 0.5 mg of milk proteins (or 0.033 ml post-heparin plasma) and varying amounts of apoLp-Glu and apoLp-Ala. Apoproteins (alone or premixed with phospholipids) and "substrate" were incubated for 30 min prior to addition of enzyme. The reactions with enzyme were stopped after 30 min. All incubations were carried out at 28°. The release of ^{14}C-labelled fatty acids was determined by the method of Kelley as modified by Huttunen et al. (16).

RESULTS

Effects of apoLp-Glu: The very marked potency of apoLp-Glu as an activator of milk lipase is shown in Fig. 1. Maximal activation was obtained at levels of 2-5 μg/ml of incubate. At levels above 200 μg/ml less than maximal activation was obtained with some preparations of apoLp-Glu. This activation was not accentuated by VLDL phospholipids or lecithin. Rather, phospholipid preparations studied to the present have reduced the potency of apoLp-Glu (Fig. 1).

Effects of apoLp-Ala: The two forms of apoLp-Ala which differ in their carbohydrate content were studied separately (17). The form containing one sialic acid moiety is designated apoLp-Ala_1 and that containing two, apoLp-Ala_2. These preparations gave activation at low levels with maximal effect usually occurring at 20 μg/ml (Fig. 2). The degree of activation was quite variable with different lots of either form of apoLp-Ala. However, levels of 100 μg/ml consistently produced inhibition of baseline enzyme activity. Addition of apoLp-Ala to assays containing adequate apoLp-Glu for maximal activation produced inhibition at levels above 25 μg/ml (50% inhibition at 60 μg/ml and over 95%

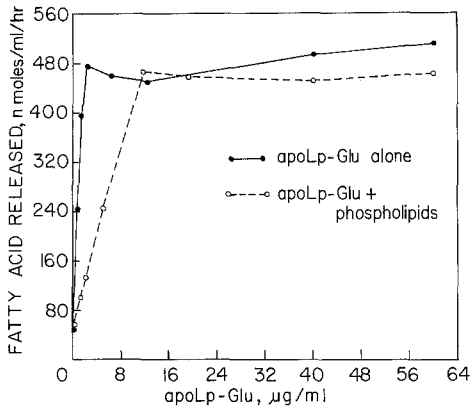

Fig. 1. Activation of LPL by apoLp-Glu. Increasing amounts of apoLp-Glu alone (●——●) or with 0.067 mg phospholipid (o— —o) were added to the assay mixture for milk LPL as described in the text.

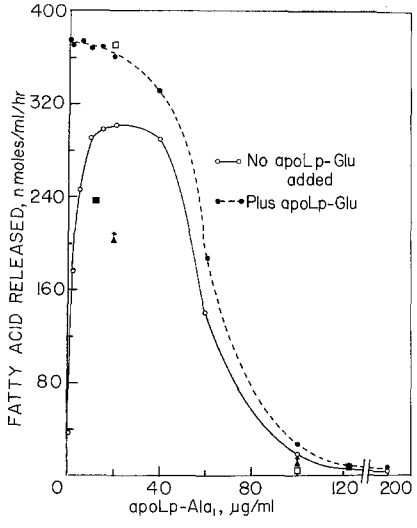

Fig. 2. Effects of apoLp-Ala$_1$ before purification on hydroxylapatite. Increasing amounts of apoLp-Ala$_1$ alone (o——o) or with a constant amount of apoLp-Glu (40 µg/ml) (●— —●) were added to the assay mixture. The points of maximal activation and of inhibition (at 100 µg/ml) are also plotted for other preparations of apoLp-Ala$_1$ (with no apoLp-Glu added). Normal pool ■ , and Type V ✱ ▲ □.

inhibition at 100 µg/ml of incubate)(Fig. 2).

Chromatography of apoLp-Ala$_1$ on hydroxylapatite (Fig. 3) separated a very small component of protein immunochemically identical with apoLp-Glu. Over 95% of the

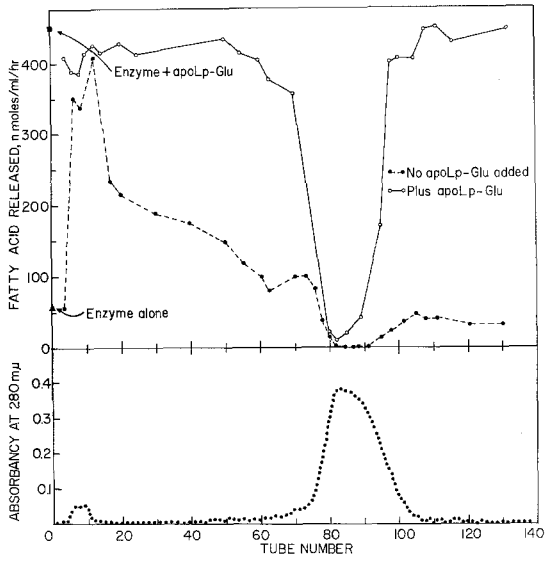

Fig. 3. Chromatography of apoLp-Ala_1 on column of hydroxylapatite. Following DEAE chromatography apoLp-Ala_1 (10 mg protein) was dialyzed versus potassium phosphate buffer (0.005 M, pH 6.9) and applied to a hydroxylapatite column (1.6 x 3.6 cm) equilibrated with the same buffer. The protein was eluted with a linear gradient of potassium phosphate from 0.01 M (300 ml) to 0.2 M (300 ml) at a flow rate of 22 ml per hr. Fractions of 2.6 ml were collected. After adjustment of pH 8.0 by addition of small volumes of 1 M dibasic potassium phosphate, 0.3 ml of the fractions indicated were added to the assay mixture. (●— —●) No apoLp-Glu added. (o——o) 10 μg/ml apoLp-Glu added. (● ● ● ●) Absorbancy at 280 mμ.

protein chromatographed as a single peak which was identified as apoLp-Ala_1 by polyacrylamide gel electrophoresis and immunochemical analysis. Activation of LPL by the first component occurred at levels of protein very similar to those observed with apoLp-Glu. The apoLp-Ala_1, after further purification on hydroxylapatite chromatography, no longer activated LPL. At levels above 25 μg/ml it inhibited the enzyme as before. Similar inhibition occurred with PHLA as the enzyme source. Addition of phospholipid in equal quantities (w/w) with the apoprotein did not alter this inhibition. Complete inhibition with 100 μg/ml was not significantly affected by addition of phospholipid up to 400 μg/ml, more enzyme (6-fold), more apoLp-Glu, more gum arabic or normal plasma. Addition of more substrate immediately reversed the inhibition. Quantities of bovine serum albumin (Fraction V) greater than those in the original "substrate" mixture also partially reduced

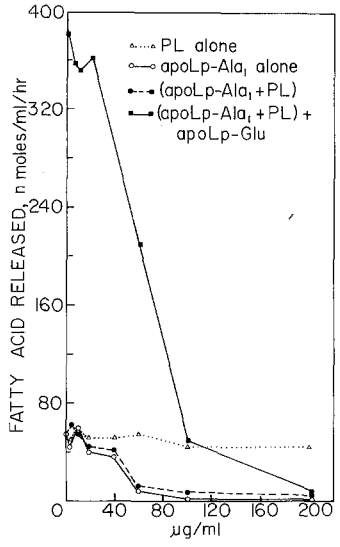

Fig. 4. Effect of apoLp-Ala$_1$ after purification on hydroxylapatite. Assays were performed with addition of phospholipid alone (Δ·····Δ), apoLp-Ala$_1$ alone (o——o), a mixture of apoLp-Ala$_1$ and equal weights of phospholipid (●– – –●), or the latter mixture plus 2 µg/ml apoLp-Glu (■——■).

the inhibition whereas fatty acid-free albumin had no effect.

No differences in degree of activation by apoLp-Glu or inhibition by apoLp-Ala$_1$ were noted when preparations of these apoproteins from normal subjects were compared to those from Type V hyperlipoproteinemics.

DISCUSSION

The present studies indicate that apoLp-Glu may act as the principal apolipoprotein activator of lipoprotein lipase in human plasma, as suggested by LaRosa et al. (3). In contrast to the data of others (3,4), our results show apoLp-Ala to be an inhibitor of LPL and to have no stimulatory activity if sufficiently pure. The activation of LPL by apoLp-Ala previously observed can now be explained by very small contamination with apoLp-Glu. The requirement of phospholipid for activation by apoLp-Ala, noted previously (3), is also consistent with apoLp-Glu contamination since phospholipid markedly potentiated the effect of apoLp-Glu. Phospholipid has been required for maximal LPL activation only with sources of LPL which have undergone organic solvent extraction (2,3).

Skim milk probably contains adequate phospholipid for potentiation of the apoLp-Glu activation and therefore no greater LPL activity was noted when these lipids were added in the present study.

Inhibition of LPL by apoLp-Ala could be explained by binding of the apoprotein to the substrate and the prevention of enzyme-substrate interaction. In the present assay, amounts of apoLp-Ala exceeding 2% of the weight of the substrate reduced the hydrolysis of triglyceride containing sufficient apoLp-Glu for maximal activation. Yields from column chromatography of these apoproteins indicate that the weight of apoLp-Ala probably exceeds 2% of the triglyceride present in the average VLDL lipoprotein. However, comparisons of the effect of these apoproteins on artificial and naturally occurring substrates will also require the consideration of substrate surface area, and the presence of other lipids and other apoproteins. The possible physiological role of an activator and an inhibitor apoprotein being associated with a substrate for lipoprotein lipase (VLDL) remains to be determined.

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