Vol. 100, No. 1, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 15, 1981 Pages 261-266

APO A-I AND APO A-II INHIBIT HEPATIC TRIGLYCERIDE LIPASE FROM HUMAN POSTHEPARIN PLASMA

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Received February 11,1981

SUMMARY

The effect of apolipoprotein A-I and A-II on hepatic triglyceride lipase activity was studied. Purification of apolipoprotein A-I and A-II was carried out by gel filtration with Sephadex G-200 in 6 M urea. Hepatic triglyceride lipase was purified from human postheparin plasma by heparin-Sepharose affinity chromatography. We demonstrated that both apolipoprotein A-I and A-II substantially inhibited hepatic triglyceride lipase. It suggests that these apolipoproteins play an important role in the inhibition of hepatic triglyceride lipase activity by whole serum.

INTRODUCTION

Postheparin plasma contains two kinds of triglyceride lipases,i.e. lipoprotein lipase (LPL) and hepatic triglyceride lipase (H-TGL) (1). Although the role of LPL on the metabolism of triglyceride-rich lipoproteins has been almost thoroughly described, the physiologic function of H-TGL is still remain to be obscure. In contrast to LPL which is activated by apo C-II (2), H-TGL does not require any plasma factor for its activation and is strongly inhibited by the addition of serum (3).

Therefore, an identification of the regulating factors of its enzyme activity in serum is thought to be of major importance. We reported in the previous paper that the addition of d=1.21 bottom fraction and high density lipoprotein (HDL) to the assay

0006-291X/81/090261-06$01.00/0

mixture caused a remarkable inhibition of H-TGL activity, which reproduced the inhibitory effect of the whole serum (4). In the present study, evidence was obtained indicating that H-TGL activity is markedly inhibited by apolipoprotein A-I (apo A-I) and apolipoprotein A-II (apo A-II) which are the major apolipoproteins of HDL.

MATERIALS AND METHODS

Purification of apo A-I and apo A-II High density lipoprotein (HDL, $1.063 < d < 1.21$) was separated from sera of normolipidemic subjects by ultracentrifugation using HITACHI RP 65 rotor (5). After dialysis against 0.195 M NaCl, HDL was delipidated with methanol-diethylether (3:2,v/v) two times and washed with diethylether three times at -10 ~ -20°C (6). The delipidated protein was dried under a stream of nitrogen and dissolved in 10 mM Tris-HCl buffer containing 6 M urea (pH 8.3). About 10 mg of protein was applied to a column of Sephadex G-200 equilibrated with the same buffer at 4°C using an ascending flow as described by Scanu et.al.(7). Fractions of the first main peak corresponding to apo A-I and the following peak corresponding to apo A-II were separately collected, concentrated and re-chromatographed with the same column for further purification. Re-chromatography for apo A-II was done twice to avoid the contamination of apo A-I. The eluted protein fractions were dialyzed against 100 mM NH_4CO_3 successively, liophylized and dissolved in 0.01 M Tris-HCl (pH 8.8). The purity of apo A-I and apo A-II was evaluated with sodium dodecyl sulfate (SDS) 10 % polyacrylamide gel electrophoresis (8).

Purification of H-TGL Hepatic triglyceride lipase was purified from the postheparin plasma obtained from healthy males ten minutes after intravenous injection of sodium heparin (13 units/kg body weight). The partial purification was carried out by means of affinity chromatography on heparin-Sepharose 4B following the method of Iverius (9). Postheparin plasma was diluted with an equal volume of 5 mM veronal-HCl buffer (pH 7.4) containing 0.45 M NaCl and applied to a column of heparin-Sepharose. After washing the column with 0.3 M NaCl-Buffer, the H-TGL was eluted with 0.75 M NaCl-Buffer.

Assay of H-TGL activity The substrate was glycerol tri[1-^{14}C] oleate (Amersham, England) diluted with unlabelled glycerol trioleate (P-L Biochemicals, Inc. Wis. USA)(S.A.=0.05 μCi/μmole). This glycerol trioleate was emulsified with gum arabic solution by Sonifier Cell Disruptor Model W185 (Heat systems-ultrasonics, Inc. N.Y. USA). The assay mixture contained 20 μl of purified H-TGL (367 μg of protein/ml), 5 μmole of glycerol trioleate, 0.5 m mole of NaCl and 5 mg of bovine serum albumin defatted by charcoal treatment (10) in 0.2 M Tris-HCl buffer (pH 8.8). The final volume of the incubation mixture was 0.5 ml. After incubation for 60 min at 27°C in a shaking bath, the reaction was terminated by adding 3.5 ml of a mixture of methanol, chloroform and n-heptane (5:5:4 by volume) and 0.75 ml of 0.1 M K_2CO_3 (11). After vigorous shaking and centrifugation at 1000 rpm for 15 min at 20°C, 0.5 ml aliquots of upper phase were transferred to scintillation vials

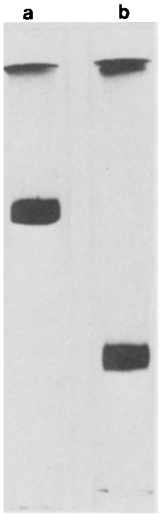

Fig.1 SDS polyacrylamide gel electrophoresis of purified apo A-I (a) and apo A-II (b) used in this study. These proteins were reduced with 2-mercaptoethanol before electrophoresis. Apo A-I and apo A-II showed a single band respectively.

and counted in a Tracor Analytic Mark III with 5 ml of toluene-PPO-POPOP scintillation fluid containing 25 % Triton X-100 and 10 % methanol.

RESULTS

Purity of apo A-I and apo A-II Figure 1 shows the SDS polyacrylamide gel electrophoresis of apo A-I and apo A-II used in this study. Each apolipoprotein was detected as a single band and the purity was thought to be satisfactory.

Identification of H-TGL The lipase activity of the protein which was eluted with 0.75 M NaCl-Buffer was not inhibited in the presence of 1 M NaCl and not activated by adding serum in vitro (data not shown). These behavior coincided with the known characteristics of H-TGL. The purified H-TGL had a specific activity of 71.4 μmole $FFA.h^{-1}.mg^{-1}$.

Effects of apo A-I and apo A-II on H-TGL activity As the activity of H-TGL measured by this assay system was linear to 30

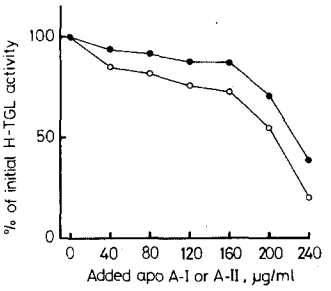

Fig.2 The effects of added apo A-I(—●—) and apo A-II (—○—) on H-TGL activity. Glycerol tri-[1-^{14}C]oleate (S.A.=0.05 μCi/μmole) was emulsified with gum arabic solution. The assay mixture contained 20 μl of purified H-TGL (367 μg of protein/ml), 5 μmole of glycerol trioleate, 0.5 mmole of NaCl, 5 mg of bovine serum albumin defatted by charcoal treatment in 0.2 M Tris-HCl buffer (pH 8.8). The final volume of incubation mixture was 0.5 ml. After incubation for 60 min at 27°C, the reaction was terminated by adding 3.5 ml of a mixture of methanol, chloroform and n-heptane and 0.75 ml of 0.1 M K_2CO_3 following the method of Belfrage and Vaugham. After vigorous shaking and centrifugation at 1000 rpm at 20°C, 0.5 ml of upper phase were counted with a scintillation counter (detail in text).

μl of the enzyme solution, we used 20 μl (367 μg of protein/ml) of H-TGL for determining the effect of apo A-I and apo A-II on H-TGL activity. H-TGL activity was markedly inhibited by apo A-I and apo A-II. The inhibition by apo A-II was more pronounced than by apo A-I. About 50 % inhibition was noted at 200 μg/ml for apo A-II compared with about 25 % inhibition at the same concentration for apo A-I (Fig.2).

DISCUSSION

Hepatic triglyceride lipase is inhibited by the addition of whole serum to incubation mixtures (3,4,12). We have demonstrated that the inhibitory effect was almost completely reproduced by the coexistance of HDL and d=1.21 bottom fraction (4). As for the effect of apolipoproteins, Kinnunen et.al. reported C-apolipoproteins inhibited H-TGL activity (3). Except for this report, there have been no studies concerning apolipoproteins

which modify the H-TGL activity. In this report, we demonstrated that the H-TGL activity was also inhibited by apo A-I and apo A-II in vitro. The meaning of these inhibition of H-TGL remains to be obscure.

Kuusi et.al. recently reported the treatment of rat with anti H-TGL resulted in an increase in cholesterol and phospholipid concentration in LDL and HDL (13). Jansen et.al. also reported an increase in phospholipid and free cholesterol concentration of HDL_2 and all components of VLDL plus IDL fractions after the treatment with anti H-TGL γ-globulins (14). These data indicate that so-called H-TGL or lipase of hepatic origin have a role for the removal of cholesterol or phospholipids from lipoproteins rather than the hydrolysis of triglyceride in vivo. But, in vitro, H-TGL actually shows triglyceride lipase activity and its triglyceride lipase activity was inhibited by serum, HDL plus d=1.21 bottom fraction, apo A-I and apo A-II. If H-TGL is located on the surface of endothelial cells of hepatic sinusoids as suggested by Kuusi et.al.(15), it always contacts with serum, d=1.21 bottom fraction, HDL and its components which inhibit the enzyme activity.

The discrepancy about the properties of so-called hepatic lipase between the results in vitro and in vivo could be explained by this inhibitory effect of apo A-I and apo A-II or whole serum on the hydrolysis of triglyceride.

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