Role of Apolipoprotein A-IV in Hepatic Lipase-Catalyzed Dolichol Acylation and Phospholipid Hydrolysis†

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ABSTRACT: Hepatic lipase catalyzes the hydrolysis of phospholipids and neutral glycerides as well as transacylation reactions between several of these lipids. We have previously reported that this enzyme also transacylates the *sn*-1 fatty acid of phosphatidylethanolamine to dolichol and that this reaction requires a plasma cofactor. In this study, we have purified the cofactor from the lipoprotein-free fraction of human plasma and present evidence demonstrating that it is identical to apolipoprotein A-IV. The effect of apolipoprotein A-IV on hepatic lipase-catalyzed dolichol acylation and phospholipid hydrolysis was studied in model membranes and compared with the effects of apolipoprotein A-I and E. Apolipoprotein A-IV strongly stimulated dolichol acylation and phosphatidylethanolamine hydrolysis but partly inhibited phosphatidylcholine hydrolysis. Apolipoprotein A-I had only a minor influence on the various activities studied and could not replace apolipoprotein A-IV. Apolipoprotein E stimulated the hydrolysis of both phospholipids but had no effect on dolichol acylation. The effect of apolipoprotein A-IV on hepatic lipase activity was then studied with the gum arabic-stabilized triglyceride emulsion. The apolipoprotein neither stimulated nor inhibited triglyceride hydrolysis in the emulsion. Finally, human high-density lipoprotein-2 and very low-density lipoprotein were also used as substrates. Apolipoprotein A-IV strongly stimulated the hydrolysis of phosphatidylcholine and phosphatidylethanolamine in both lipoproteins, while the hydrolysis of triglycerides was completely inhibited. These results demonstrate that apolipoprotein A-IV is an important cofactor to hepatic lipase affecting both catalytic rates and the substrate specificity of the enzyme. We therefore suggest that apolipoprotein A-IV-rich high-density lipoprotein is the preferred substrate for hepatic lipase.

Hepatic lipase (HL)[1] is present in the liver of most mammals and has also been detected in adrenal glands and ovaries (Jansen & De Greef, 1981). In liver, the enzyme is mainly located on the surface of endothelial cells (Kuusi et al., 1979), but high activity has also been found in hepatocytic endosomes (Hornick et al., 1992). Studies *in vitro* indicate that HL can hydrolyze both triglycerides and phospholipids in lipoproteins (Deckelbaum et al., 1992) and that it possesses high affinity for HDL particles (Bengtsson & Olivecrona, 1980). From experiments *in vivo*, it appears that HL is involved in the catabolism of chylomicron remnants (Shafi et al., 1994), the conversion of IDL to LDL (Goldberg et al., 1982), and the conversion of HDL-2 to HDL-3 and pre-β_1 HDL (Jansen et al., 1980; Tikkanen et al., 1982; Barrans et al., 1994). HL-catalyzed hydrolysis of HDL phospholipids has also been shown to be coupled to the selective uptake of HDL cholesteryl esters by the liver (Kadowaki et al., 1992; Marques-Vidal et al., 1994). This function could be an important part of reverse cholesterol transport, but the mechanism remains unclear.

HL deficiency in humans is characterized by elevated plasma levels of triglycerides and cholesterol with resultant premature atherosclerosis (Connelly et al., 1990; Hegele et al., 1993). In addition, overexpression of HL in transgenic rabbits results in a marked reduction of plasma cholesterol and triglycerides in mainly HDL and IDL (Fan et al., 1994). These findings clearly emphasize the importance of HL in lipoprotein metabolism, but the exact role of HL has not been established.

The effects of various apolipoproteins on HL activity have been extensively studied using gum arabic-emulsified triglycerides and chylomicrons as substrates (Jackson, 1993; Kinnunen, 1984). All apolipoproteins tested inhibited triglyceride hydrolysis, and this effect was attributed to a steric hindrance of the enzyme binding. In an attempt to better characterize the true nature of the lipoprotein substrate, Thuren et al. (1991a) reinvestigated the effects of apolipoproteins (apo) A-I, A-II, C-I, C-II, C-III, and E on HL hydrolysis in lipid monolayers. With the exception of apo E, all apolipoproteins inhibited both phospholipid and triglyceride hydrolysis. Apo E mainly stimulated phospholipid hydrolysis, and this stimulation was dependent on low surface pressure. In a later publication, they also demonstrated that HL had higher activity toward apo E-rich HDL than toward apo E-poor HDL and that phosphatidylcholine (PC) was the preferred substrate (Thuren et al., 1992).

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[1] Abbreviations: HL, hepatic lipase; PEDAT, phosphatidylethanolamine:dolichol acyltransferase; LCAT, lecithin−cholesterol acyltransferase; apo, apolipoprotein; HDL, high-density lipoprotein; VLDL, very low-density lipoprotein; PC, phosphatidylcholine; PE, phosphatidylethanolamine; TG, triglycerides; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis.

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Dolichols are polyisoprenoid lipids containing a primary alcohol group which can be esterified with either a fatty acid or a phosphate (Chojnacki & Dallner, 1988). Present in all organs and cellular membranes, this lipid is believed to be localized in the central hydrophobic portion of membranes, sandwiched between the two halves of the phospholipid bilayer. In model systems, dolichol destabilizes membranes and increases permeability as well as phospholipid fatty acid fluidity (de Ropp & Troy, 1985; Valtersson et al., 1985). It also displays fusogenic properties and may therefore play a role in membrane trafficking and membrane−lipoprotein interactions. Like cholesterol, dolichol can be esterified with a CoA-activated fatty acid by a specific enzyme in microsomes (Tollbom et al., 1988).

We have previously shown that HL, in addition to its hydrolytic activities, also possesses a dolichol-acylating activity (Sindelar et al., 1992; Sindelar & Valtersson, 1993). In this reaction, the fatty acid in the *sn*-1 position of phosphatidylethanolamine (PE) is transacylated to dolichol (PEDAT activity). We also presented evidence for the existence of a plasma cofactor that strongly activates this reaction and shifts the pH optimum from 8.5 to 7.5. When human plasma was fractionated by gel filtration, the majority of this cofactor was found in the molecular weight range of HDL. However, HDL isolated by ultracentrifugation on a discontinuous KBr gradient did not stimulate the reaction, and the cofactor was found in the lipoprotein-free fraction.

Here, we report the isolation and characterization of this plasma cofactor and demonstrate that it is identical to apo A-IV. We also compare the effects of apo A-IV with those of apo A-I and apo E on HL-catalyzed dolichol acylation and phospholipid hydrolysis in model membranes. Finally, we show that apo A-IV is an important cofactor to HL during the hydrolysis of lipoproteins.

MATERIALS AND METHODS

Lipids. Dolichol-19 was isolated from autopsy specimens of human liver by reversed-phase chromatography (Mankowski et al., 1976) and was labeled with [^{3}H]sodium borohydride (57 Ci/mmol, Amersham) according to Keenan and Kruczek (1976). Upon thin-layer chromatography in different solvent systems, [^{3}H]dolichol-19 migrated as a single spot.

Proteins. HL used in this report was partially purified from heparinized rat liver perfusates according to Sindelar et al. (1992). The enzyme was stored in 50% glycerol at −80 °C.

Human apo A-I and apo A-IV were purified from the lipoprotein-free fraction (d > 1.25 g/mL) of plasma by the procedure of Weinberg and Spector (1985) with minor modifications. In order to extract the apolipoproteins from the d > 1.25 g/mL fraction, we used 15% Intralipid instead of 10%. The ion exchange chromatography step was performed on a DEAE-Sephacel column using a linear gradient of 40 to 120 mM Tris-HCl in 7 M urea at pH 8.3. The absorption of the eluate was monitored at 280 nm, and the fractions obtained were analyzed by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) (Laemmli, 1970) and silver staining (Bio-Rad). Apo A-I eluted as a sharp peak at approximately 70 mM Tris-HCl and apo A-IV as a broad peak around 100 mM. Complete baseline separation between the two proteins was obtained in this step. The purified apolipoproteins were dialyzed against 1 mM Tris-HCl at pH 7.4 until the concentration of urea dropped below 0.1 M and then were concentrated and desalted by repetitive centrifugations using Centriprep concentrators. Finally, the concentrated solutions were extensively dialyzed against 5 mM NH_4HCO_3 at pH 8.0 and stored in aliquots at −20 °C. Human apolipoprotein E was purchased from Calbiochem.

Isolation of Human Plasma and Lipoproteins. Fresh blood was collected from fasting healthy normolipidemic volunteers in tubes containing EDTA (1 mg/mL). Plasma was immediately separated at 4 °C by low-speed centrifugation. VLDL (d < 1.006 g/mL) was isolated by sequential salt (KBr) density ultracentrifugation in a Beckman 50.2 rotor (42 000 rpm, 22 h) at 4 °C as described previously (Schumaker & Puppione, 1986). HDL-2 (d = 1.063−1.125 g/mL) was isolated after 42 h of ultracentrifugation at 40 000 rpm. The isolated lipoproteins were extensively dialyzed against 150 mM NaCl, 10 mM Tris-HCl, 0.01% azide, and 0.01% EDTA at pH 7.5 with a minimum of three changes of dialysis buffer and stored under nitrogen in the dark at 4 °C.

Preparation of Apo A-IV Antibodies. New Zealand white rabbits (2.5−3 kg) were immunized with 150 μg of purified human apo A-IV. The protein solution was emulsified with an equal volume of Freund's complete adjuvant and injected intradermally into multiple sites on the back of the rabbits. Booster immunizations were performed with 200 μg of the antigen at 2 week intervals until the serum yielded a strong immunoprecipitate line against apo A-IV on Ochterlony plates. The rabbits were bled by the ear vein, and the antiserum was fractionated by ammonium sulfate precipitation followed by chromatography on a cellulose anion exchanger (DE53, Whatman). The resulting γ-globulin fraction was dialyzed against 150 mM NaCl and 10 mM Tris-HCl at pH 7.3 and stored in the presence of 0.01% azide at 4 °C. The antibodies were monospecific toward apo A-IV as judged by Western blots of human plasma.

Dolichol Acylation and Phospholipid Hydrolysis in Model Membranes. Determination of HL-catalyzed dolichol acylation was performed with small unilamellar liposomes consisting of dioleoyl-PC/dioleoyl-PE (3:1) and 2 mol % [^{3}H]-dolichol-19 (330 mCi/mmol), as described previously (Sindelar & Valtersson, 1993). When apolipoproteins and fractions from the gel filtration experiment were tested for regulatory effects on the enzyme, plasma was omitted from the assay mixture.

The same liposomes were used for detection of HL-catalyzed hydrolysis of PE and PC except that dolichol was omitted. The reaction mixture (1 mL) contained 0.2 M Tris-HCl (pH 7.5), 10 mM $CaCl_2$, 2.5 mg of bovine serum albumin, 5 μmol of lipid, and the enzyme. All incubations were conducted at 37 °C for 50 min. The reaction was stopped by adding 5 mL of methanol, 10 mL of chloroform, and 4 mL of H_2O. The tubes were tightly sealed with stoppers, vigorously stirred on a vortex, and centrifuged at low speed to obtain phase separation. The chloroform phase was transferred and evaporated to dryness with a stream of nitrogen gas. The residue was redissolved in 100 μL of chloroform, and an aliquot was applied to a Silica Gel 60 plate (Merck). The mobile phase was chloroform/methanol/ammonia (65:25:5), and lipids were detected with iodine vapor. The gel containing lyso-PC and lyso-PE was scraped off, transferred to acid-washed glass tubes, and subjected to

hydrolysis in 1.0 mL of perchloric acid at 140 °C for 2 h. After cooling and centrifugation at 2000 rpm for 15 min, phosphate determination was performed as follows. Hydrolysate (300 μL) was transferred to acid-washed glass tubes to which 1 mL of H_2O, 400 μL of 1.25% ammonium molybdate, and 400 μL of 5% ascorbic acid (freshly prepared) were added. The tubes were incubated in a boiling water bath for 30 min, and the extinction at 797 nm was subsequently measured using monobasic potassium phosphate as the standard.

Assay of Triglyceride Hydrolysis. Triglyceride hydrolysis was determined using a gum arabic-stabilized emulsion of tri[1-^{14}C]oleoylglycerol (120 mCi/mmol, New England Nuclear) in the presence of 1 M NaCl as described earlier (Ehnholm & Kuusi, 1986). Incubation was conducted at 32 °C for 30 min.

Phospholipid and Triglyceride Hydrolysis in Human HDL-2 and VLDL. The hydrolysis reaction mixture consisted of 50 mM Tris-HCl (pH 7.6), 150 mM NaCl, 2 mM $CaCl_2$, and 1% bovine serum albumin in a total volume of 1.0 mL. Concentrations of HDL-2 and VLDL were 1.2 and 2.6 mg of protein/mL, respectively. The reaction was initiated by the addition of 0.3 μg of partially purified HL and allowed to proceed for 2.5 h at 37 °C. The incubation was terminated by adding 5 mL of methanol and 10 mL of chloroform, followed by 4 mL of H_2O. Before the extraction procedure, 0.1 μCi [*choline-methyl*-^{14}C]phosphatidylcholine and 0.1 μCi [*carboxyl*-^{14}C]triolein (New England Nuclear) were added to the mixtures as internal standards. After phase separation, the chloroform phase was loaded on a column (0.5 × 2 cm) of silicic acid (Merck). Neutral lipids were eluted with chloroform and phospholipids with methanol. The phospholipids were separated by thin-layer chromatography and quantitated by phosphate determination. The neutral lipids in the chloroform eluate were evaporated to dryness, dissolved in 1 mL of hexane, and applied to a second column (0.5 × 2 cm) of silicic acid equilibrated in hexane. To separate monoglycerides and diglycerides from the triglycerides, the column was first washed with hexane and then with 1% diethyl ether in hexane. Triglycerides were eluted with 3% diethyl ether in hexane, and the eluate was evaporated and dissolved in 250 μL of acetone. Quantitation of triglyceride content was performed using a commercial triglyceride kit (Sigma). The yields of phospholipids and triglycerides were 70−80%.

Gel Filtration Experiment. Fresh human plasma (3 mL) was applied to a 1.6 × 80 cm column of Bio-Gel A-1.5 (Bio-Rad) equilibrated in 0.15 M NaCl, 10 mM Tris-HCl, 5 mM EDTA, and 0.02% azide at pH 7.4. The flow rate was 0.11 mL/min, and fractions of 1.65 mL were collected. A standard gel filtration kit from Bio-Rad was used for column calibration. Each fraction (300 μL) was tested for its ability to stimulate HL-catalyzed dolichol acylation. The concentration of apo A-IV was measured by a noncompetitive ELISA. The samples were diluted 1:100 with 0.05 M carbonate−bicarbonate buffer at pH 9.6, and 50 μL was plated into each well (polystyrene microwell plates, Maxisorp C, Nunc). After overnight incubation at 4 °C, the plate was washed four times with a buffer consisting of 0.05 M Tris-HCl (pH 7.6), 0.9% NaCl, 0.1% Tween-20, and 1% bovine serum albumin. Rabbit anti-apo A-IV IgG was diluted to 0.75 μg/mL in the same buffer, and 100 μL was pipetted into each well. After 45 min at 37 °C, the plate was washed several times and bound antibodies were detected using alkaline phosphatase-conjugated anti-rabbit antibodies (Sigma) and *p*-nitrophenyl phosphate. Apo A-I was measured using an immunoturbidimetric kit from Boehringer Mannheim.

RESULTS

Purification of Cofactor. HL catalyzes a transacylation from PE to dolichol, and this reaction is strongly stimulated by a plasma cofactor (Sindelar et al., 1992; Sindelar & Valtersson, 1993). Upon gel filtration of human plasma on Bio-gel A-1.5, the stimulatory activity was heterogeneously distributed with a dominating peak around 220 kDa, which indicated that the majority of the cofactor is associated with HDL. However, when lipoproteins were separated by sequential salt density ultracentrifugation, no stimulatory effect could be achieved with the isolated lipoproteins and the cofactor was only found in the lipoprotein-free fraction (d > 1.25 g/mL). These results suggested that the cofactor was bound to HDL in whole plasma but dissociated completely from this lipoprotein upon ultracentrifugation. Only one apolipoprotein is known to behave in this manner, namely apo A-IV (Bisgaier et al., 1985; Lagrost et al., 1989), and we therefore purified this apolipoprotein according to the method of Weinberg and Spector (1985). All fractions obtained during the purification procedure were tested for stimulation of the HL-catalyzed dolichol acylation and analyzed for protein composition by SDS−PAGE and silver staining. The final preparation obtained after anion exchange chromatography contained a single protein band with a molecular mass of 46 kDa corresponding to apo A-IV. In all purification steps, only those fractions that contained apo A-IV exhibited stimulatory activity, and thus, the purification of this protein could be conveniently followed with the assay for dolichol acylation. The amount of apo A-IV obtained from 280 mL of plasma was 2.9 mg, and this preparation was subsequently used to characterize the stimulatory effect of apo A-IV and to raise antibodies against this protein in rabbits.

Distribution of the Stimulatory Activity and Apo A-IV upon Gel Filtration of Human Plasma. In Figure 1, fractions from the gel filtration of human plasma on Bio-gel A-1.5 were analyzed for the presence of apo A-I and apo A-IV, and the stimulation of the HL-catalyzed dolichol acylation. Apo A-I appeared as a symmetrical peak around 190 kDa and corresponds to the elution of HDL. The distribution of apo A-IV was measured with a noncompetitive ELISA and revealed a major peak around 220 kDa and a shoulder in approximately the same molecular mass range as that for serum albumin (68 kDa). This behavior of apo A-IV upon gel filtration of whole plasma is in accordance with earlier observations where the majority of the apolipoprotein was bound to a subpopulation of HDL with a somewhat higher molecular mass, while a smaller portion was found in the low-molecular mass range as free apo A-IV (Lagrost et al., 1989). As can be seen from this figure, the stimulation of dolichol acylation catalyzed by HL is superimposed on the distribution of apo A-IV.

We also tried to remove the stimulatory activity from whole plasma with the antibodies against apo A-IV. These attempts were, however, unsuccessful because only about 35% of the total apo A-IV in plasma was bound by the antibodies in the absence of detergent (results not shown).

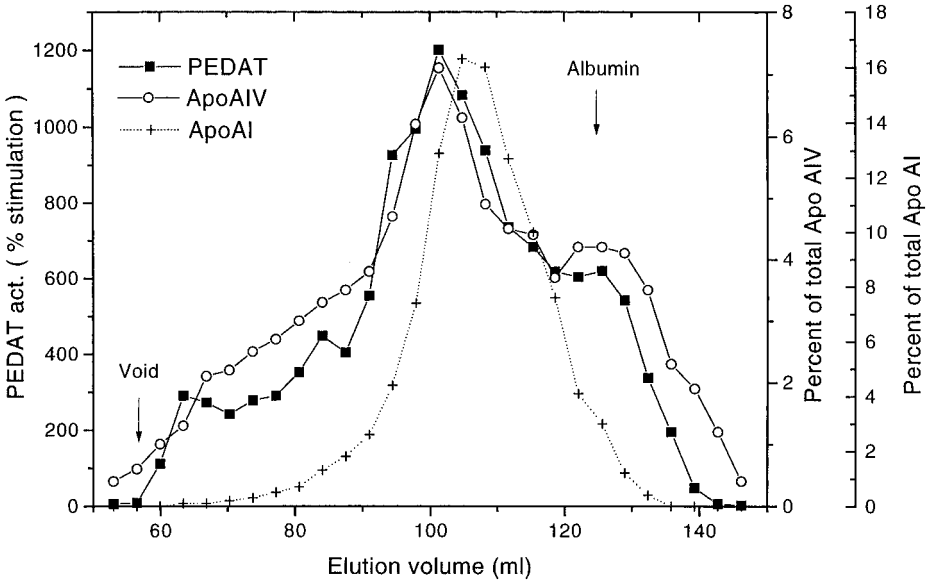

FIGURE 1: Distribution of the PEDAT cofactor, apo A-IV, and apo A-I after gel filtration of human plasma on Bio.Gel A-1.5. Fractions were collected and analyzed as described in Materials and Methods. The total sum of all values for each apolipoprotein represents 100%. The values are means of duplicate samples from a representative fractionation.

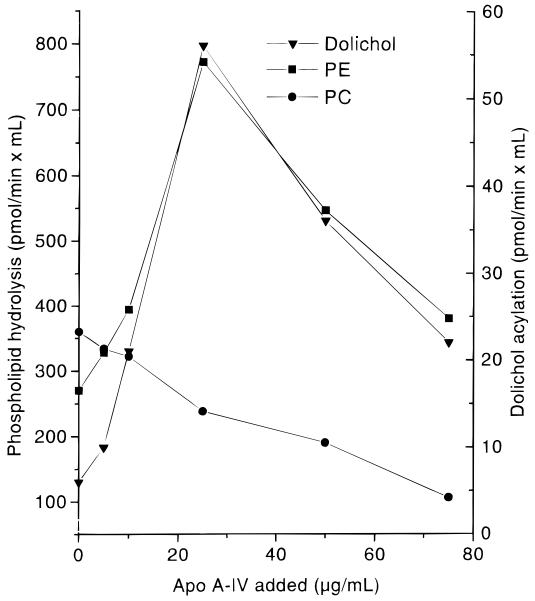

FIGURE 2: Effect of apolipoprotein A-IV on dolichol acylation and phospholipid hydrolysis by hepatic lipase in model membranes. The assay was performed with 0.1 μg of partially purified enzyme under standard assay conditions in the absence and presence of increasing concentrations of apolipoprotein A-IV. Phospholipid hydrolysis is expressed as picomoles of lyso compounds formed and dolichol acylation as picomoles of dolichyl esters formed per minute per milliliter. The results shown are means of six different experiments.

Effects of Apolipoproteins A-I, A-IV, and E on Dolichol Acylation and Phospholipid Hydrolysis in Model Membranes. In the following experiments, we determined the effects of various concentrations of apo A-IV on the HL-catalyzed dolichol acylation and phospholipid hydrolysis in model membranes (Figure 2). In the case of dolichol acylation, the liposomes consisted of dioleoyl-PC/dioleoyl-PE (3:1) and 2 mol % dolichol-19. When phospholipid hydrolysis was measured, the acyl acceptor was omitted and albumin was added to the assay. Apo A-IV strongly activated both dolichol acylation and PE hydrolysis up to a concentration of 25 μg/mL. When the concentration was increased above this value, both activities declined quite sharply. In contrast, the hydrolysis of PC was increasingly inhibited as the concentration of apo A-IV was raised.

Apo E has been implicated as a cofactor to HL (Thuren et al., 1991a, 1992), and the enzyme shows high affinity for HDL in which apo A-I is the most abundant protein (Bengtsson & Olivecrona, 1980). We therefore compared the effects of these apolipoproteins with those of apo A-IV. Titrations of the various apolipoproteins were performed to establish optimal conditions for each reaction, and the results are summarized in Figure 3. With apo A-IV, the activation of dolichol acylation was 10-fold, while both apo A-I and apo E had marginal effects (Figure 3A). In the case of PE hydrolysis, apo A-IV had the strongest stimulatory effect and activated the reaction 3-fold (Figure 3B). Apo A-I and apo E had a less pronounced effect and stimulated the hydrolysis only 1.4- and 1.6-fold, respectively. A completely different picture was obtained when the hydrolysis of phosphatidylcholine was studied (Figure 3C). Both apo A-I and apo A-IV partially inhibited the reaction, while apo E increased the hydrolysis 1.5-fold. The specific activities of the partially purified HL in the absence of apolipoproteins were 2.8 μmol of dolichyl ester, 140 μmol of lyso-PE, and 168 μmol of lyso-PC per 50 min and per milligram of protein. Less than 10% of each lipid was acylated or hydrolyzed under the assay conditions employed, and thus, the substrate concentrations were in excess.

Effect of Apo A-IV on Hydrolysis of Gum Arabic-Stabilized Triglycerides. Regulation of HL by apolipoproteins has been extensively studied with various triglyceride substrates, but apo A-IV has never been tested (Jackson, 1983; Kinnunen, 1984). Most of the apolipoproteins either inhibited or influenced the hydrolysis to a limited extent. In agreement with previous reports, we also found an inhibition with apo A-I (10−200 μg/mL), while apo E (10−60 μg/mL) had a minor stimulatory effect (Kubo et al., 1981; Thuren et al., 1991b). Upon addition of various concentrations of purified apo A-IV (10−90 μg/mL) to the assay mixture, neither activation nor inhibition could be observed (data not shown).

Effect of Apo A-IV on the Hydrolysis of Human HDL-2 and VLDL. Apo A-IV is more weakly associated with

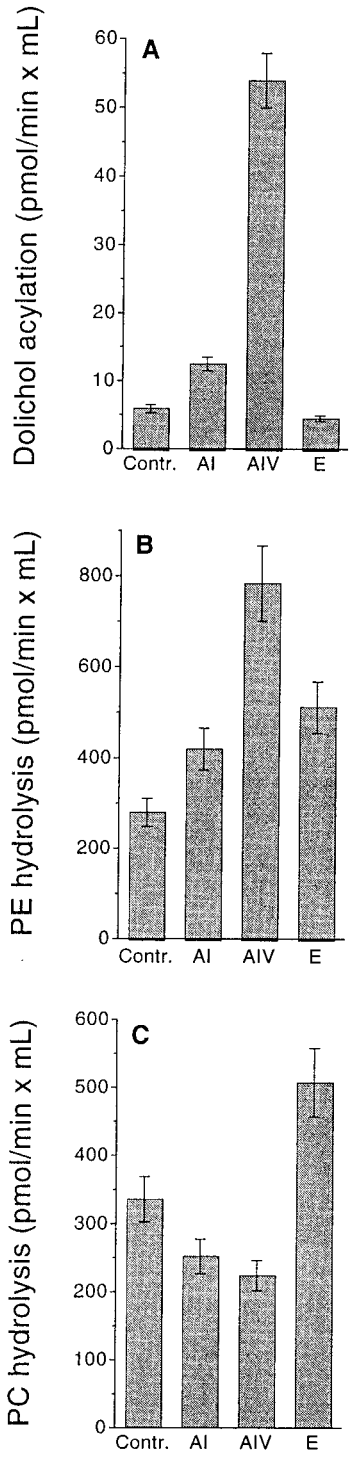

FIGURE 3: Effects of various apolipoproteins on dolichol acylation (A), PE hydrolysis (B), and PC hydrolysis (C) by hepatic lipase in model membranes. The assay was performed with 0.1 μg of partially purified enzyme (Sindelar et al., 1992) under standard assay conditions. The various apolipoproteins were titrated, and the optimal concentrations obtained were used in the individual experiments. These were 25 μg/mL for apo A-I, 25 μg/mL for apo A-IV, and 50 μg/mL for apo E. The values are means ± SD (n = 4).

lipoproteins than other apolipoproteins, and when HDL is isolated by ultracentrifugation, all apo A-IV is lost into the lipoprotein-free fraction (Bisgaier et al., 1985; Lagrost et al., 1989). Thus, the HDL-2 and VLDL used in these experiments contained no apo A-IV. Since the concentration of dolichol in lipoproteins is low and most of it acylated (Elmberger et al., 1988), we only studied the effects of apo A-IV on HL-catalyzed phospholipid and triglyceride hydrolysis. Optimal amounts of apo A-IV were determined, and radioactive PC and triolein were used as internal standards to compensate for losses during lipid analysis.

Table 1: Hydrolysis of Human HDL-2 by Hepatic Lipase[a]

substrate	control	plus apo A-IV
PC	947 ± 103[b]	1763 ± 186
PE	123 ± 10	417 ± 37
TG	383 ± 37	0

[a] The experiments were conducted as described in Materials and Methods in the absence or presence of 25 μg of apo A-IV. The values are means ± SD (n = 4). [b] Activities are expressed as picomoles per minute per microgram of partially purified hepatic lipase.

Table 2: Hydrolysis of Human VLDL by Hepatic Lipase[a]

substrate	control	plus apo A-IV
PC	942 ± 93[b]	1602 ± 123
PE	77 ± 6	203 ± 20
TG	1283 ± 130	0

[a] The experiments were conducted as described in Materials and Methods in the absence or presence of 25 μg of apo A-IV. The values are means ± SD (n = 4). [b] Activities are expressed as picomoles per minute per microgram of partially purified hepatic lipase.

Table 1 shows the hydrolysis rates of PC, PE, and triglycerides by HL in HDL-2 in the absence and presence of apo A-IV. Under both incubation conditions, more PC was hydrolyzed relative to PE and triglycerides. In the presence of apo A-IV, the rates of PC and PE hydrolysis increased 1.9- and 3.4-fold, respectively. In contrast, the hydrolysis of triglycerides was completely inhibited by apo A-IV.

When VLDL was used as a substrate in the absence of apo A-IV, the rate of triglyceride hydrolysis was about 3-fold higher than in HLD-2, while the rates of PC and PE hydrolysis were about the same (Table 2). Upon addition of apo A-IV, the hydrolysis of PC and PE increased 1.7- and 2.6-fold, respectively. As in HDL-2, the triglyceride hydrolysis in VLDL was completely inhibited by apo A-IV.

DISCUSSION

The HL-catalyzed dolichol acylation is stimulated up to 15-fold by plasma. In this study, we have isolated the cofactor responsible for this activation and demonstrated that it is identical to apo A-IV. This conclusion is based on the following results. (1) The cofactor is mainly present in an HDL-bound form in human plasma upon gel filtration but is completely released from this lipoprotein upon KBr density ultracentrifugation. Although apo A-I and apo E can also be recovered in the lipoprotein-free fraction (d > 1.25 g/mL), only apo A-IV dissociates completely from lipoproteins (Blum et al., 1980; Kunitake & Kane, 1982; Bisgaier et al., 1985). (2) The cofactor was purified to homogeneity from the lipoprotein-free fraction according to a previously published procedure for the purification of apo A-IV (Weinberg & Spector, 1985). Stimulation of dolichol acylation was only obtained with those fractions which contained this protein, and thus, all other plasma components could be excluded. (3) Finally, upon gel filtration of whole plasma, the stimulatory activity of the various fractions overlapped with the distribution of apo A-IV.

Apo A-IV very efficiently stimulates HL-catalyzed dolichol acylation and could not be replaced by either apo A-I

or apo E. The activation, however, is not restricted to this reaction alone but also involves PE hydrolysis in liposomes and, most significantly, PE and PC hydrolysis in HDL-2 and VLDL. On the other hand, the HL-catalyzed triglyceride hydrolysis of these lipoproteins is completely inhibited by apo A-IV. These findings with natural lipoprotein substrates clearly demonstrate that apo A-IV is an important cofactor to HL and greatly affects the substrate specificity of the enzyme.

There is ample evidence that the physicochemical state of the substrate has a great impact on various lipase activities. Surface charge and pressure as well as the curvature of the lipid layer are all likely to affect both the substrate specificity and cofactor dependence of the enzyme. Thuren et al. (1991a,b) have previously demonstrated that apo E stimulates HL-catalyzed phospholipid hydrolysis in monolayers and Triton X-100 micelles. Our experiments with apo E are in agreement with these findings, since this apolipoprotein stimulated both PC and PE hydrolysis in small unilamellar liposomes. Apo E, however, had no effect on dolichol acylation. They also showed that HL-catalyzed PC hydrolysis is higher in apo E-rich HDL than in apo E-poor HDL (Thuren et al., 1992). These two lipoprotein populations, however, also differed in size, protein content, and lipid composition, and it therefore remains to be established that apo E is the sole factor responsible for the observed difference. In this context, it is of interest that we used human HDL-2 and VLDL, both of which contain appreciable amounts of apo E (Blum et al., 1980; Havel et al., 1980), and yet upon addition of apo A-IV obtained significant stimulation of HL-catalyzed phospholipid hydrolysis. It is therefore very probable that, in the absence of apo E, the degree of stimulation would be considerably higher.

The ability of HL to penetrate phospholipid layers is quite weak as compared to that of other lipases (Jackson et al., 1986; Thuren et al., 1991a). Yet, dolichol is thought to be situated between the fatty acids in the deep hydrophobic portion of the phospholipid bilayer (de Ropp & Troy, 1985; Valtersson et al., 1985), and the *sn*-1 ester bond of phospholipids is also more deeply buried than the *sn*-2 bond (De Bony & Dennis, 1981). Thus, one of the stimulatory functions of apo A-IV in this reaction could be to assist the enzyme in penetrating the bilayer and allowing access to both substrates. Since apo A-IV also increases the preference of HL for PE as compared to that for PC and stimulates dolichol acylation several-fold more than PE hydrolysis, it is plausible that more specific interactions occur between the cofactor and the enzyme. This reasoning is in analogy with the stimulatory effects of apo A-I and apo A-IV on cholesterol esterification by lecithin−cholesterol acyltransferase (LCAT) (Fielding et al., 1972; Steinmetz & Utermann, 1985). This activation is dependent on the amphipathic α-helical sequences of these apolipoproteins (Jonas, 1991), which help the enzyme to interact with the lipid surface, and more specific regions, which interact with the enzyme (Banka et al., 1991; Emmanuel et al., 1994). It is not likely, however, that these "LCAT specific regions" are responsible for the activation of HL-catalyzed dolichol acylation, since apo A-I could not replace apo A-IV in this reaction.

The dolichol pool upon which HL acts *in vivo* has not been defined. Dolichol in plasma is found exclusively in HDL and mainly in an acylated form (Elmberger et al., 1988). This pool is, however, very small and its physiological role unclear, since no redistribution between different organs occurs (Chojnacki & Dallner, 1988). On the other hand, HL is situated on the plasma membranes of liver sinusoids (Kuusi et al., 1979), and these membranes are relatively rich in dolichol (Eggens et al., 1983). Thus, although the function for such dolichol acylation is unknown, the prerequisites for the reaction to occur *in vivo* are theoretically fulfilled.

Several observations suggest that apo A-IV plays an important role in the reverse cholesterol transport process. It promotes cholesterol efflux from several extrahepatic cells (Steinmetz et al., 1990; Hara et al., 1992) and activates LCAT. Upon LCAT-induced cholesterol esterification in whole plasma, lipoprotein-free apo A-IV redistributes to an HDL subpopulation with a high degree of LCAT modification (DeLamatre et al., 1983; Lefevre et al., 1989). Our results indicate that this apo A-IV-rich HDL could be the preferred substrate for HL. It is reasonable to speculate that this lipoprotein subpopulation delivers cholesteryl esters to the liver by a different route than apo E-rich HDL, which interacts with the LDL receptor (Havel & Hamilton, 1988). Thus, since the HL-catalyzed phospholipid hydrolysis of HDL is implicated in the selective uptake of cholesteryl esters by the liver (Kadowaki et al., 1992; Marques-Vidal et al., 1994), apo A-IV could be an important cofactor in this process. We are currently investigating this possibility in a liver perfusion system.

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