Activation of Lecithin:Cholesterol Acyltransferase by Human Apolipoprotein A-IV*

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Human plasma apoproteins (apo) A-I and A-IV both activate the enzyme lecithin:cholesterol acyltransferase (EC 2.3.1.43). Lecithin:cholesterol acyltransferase activity was measured by the conversion of [4-14C]cholesterol to [4-14C]cholesterol ester using artificial phospholipid/cholesterol/[4-14C]cholesterol/apoprotein substrates. The substrate was prepared by the addition of apoprotein to a sonicated aqueous dispersion of phospholipid/cholesterol/[4-14C]cholesterol. The activation of lecithin:cholesterol acyltransferase by apo-A-I and -A-IV differed, depending upon the nature of the hydrocarbon chains of the sn-1-ω-phosphatidylcholine acyl donor. Apo-A-I was a more potent activator than apo-A-IV with egg yolk lecithin, L-ω-dioleoylphosphatidylcholine, and L-ω-phosphatidylcholine substituted with one saturated and one unsaturated fatty acid regardless of the substitution position. When L-ω-phosphatidylcholine esterified with two saturated fatty acids was used as acyl donor, apo-A-IV was more active than apo-A-I in stimulating the lecithin:cholesterol acyltransferase reaction. Complexes of phosphatidylcholines substituted with two saturated fatty acids served as substrate for lecithin:cholesterol acyltransferase even in the absence of any activator protein. Essentially the same results were obtained when substrate complexes (phospholipid-cholesterol-[4-14C]cholesterol-apoprotein) were prepared by a detergent dialysis procedure. Apo-A-IV-L-ω-dimyristoylphosphatidylcholine complexes thus prepared were shown to be homogeneous particles by column chromatography and density gradient ultracentrifugation. It is concluded that apo-A-IV is able to facilitate the lecithin:cholesterol acyltransferase reaction in vitro.

Apolipoprotein A-IV (apo-A-IV) is a Mr 46,000 glycoprotein that was first demonstrated in rats (1) but was later also observed in other species, including humans (2–6). The apoprotein is synthesized as a preprotein (7, 8) primarily in mucosal cells of the small intestine and is assembled into chylomicrons during fat absorption and secreted into the lymph (6, 9). Mesenteric lymph chylomicrons from rats and lymph chylomicrons isolated from humans with chyluria or chylous ascites contain apo-A-IV together with apoproteins A-I and B-48 (3–6, 10–12). When primary chylomicrons enter the plasma compartment, they undergo rapid changes, including the loss of apo-A-IV and apo-A-I in exchange for apo-E and -C apoproteins (10, reviewed in Ref. 13). The fate of the apo-A-IV in plasma appears to be different in rats and humans. Whereas about 50% of apo-A-IV in rat plasma is associated with high density lipoproteins, more than 95% of apo-A-IV is found unassociated with the major plasma lipoproteins in fasting humans (1, 4–6, 14). No specific function has yet been assigned to apo-A-IV, and its significance in lipoprotein metabolism has remained unclear. We show here that apo-A-IV in vitro does act as an activator for the plasma enzyme lecithin:cholesterol acyltransferase (EC 2.3.1.43).

This enzyme catalyzes the transacylation from the β-position of phosphatidylcholine (PC) to the hydroxyl group of cholesterol, resulting in the formation of lysolecithin and cholesteryl ester (15, 16). This reaction seems to be a prerequisite for "reversed" cholesterol transport, e.g. the transport of cholesterol from peripheral cells to the liver (17). In the absence of an acyl acceptor, lecithin:cholesterol acyltransferase exhibits phospholipase activity (18). A further reported activity of the enzyme is that of a lysolecithin acyltransferase which requires the presence of low density lipoproteins (19, 20).

Most investigations on the specificity and regulation of the enzyme reaction have focused on the acyltransferase activity. This reaction requires a protein cofactor first identified as apo-A-I (21). In the present report, the conditions under which apo-A-IV does serve as an activator for the lecithin:cholesterol acyltransferase-catalyzed transacylation reaction have been defined in comparison to apo-A-I.

EXPERIMENTAL PROCEDURES

Materials

Egg yolk L-ω-phosphatidylcholine, L-ω-didecanoylphosphatidylcholine (DDPC), L-ω-1-oleoyl-2-palmitoylphosphatidylcholine, and L-ω-1-palmitoyl-2-oleoylphosphatidylcholine, and L-ω-1-palmitoyl-2-oleoylphosphatidylcholine were obtained from Sigma Chemie GmbH, Taufkirchen, West Germany. [4-14C]Cholesterol was purchased from New England Nuclear, Dreieich, West Germany. Lecithin:cholesterol acyltransferase and apolipoproteins were isolated from fresh plasma of healthy blood donors.

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The abbreviations used are: apo, apolipoprotein; HDL, high density lipoprotein; PC, phosphatidylcholine; SDS, sodium dodecyl sulfate; BSA, bovine serum albumin; DDPC, L-ω-didecanoylphosphatidylcholine; DMPC, L-ω-dimyristoylphosphatidylcholine; DSPC, L-ω-dipalmitoylphosphatidylcholine; DOPC, L-ω-dioleoylphosphatidylcholine.
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**Methods**

**Apporotins**—Apolipoproteins A-I and A-II were isolated essentially as outlined by Cheung and Albers (22) with minor modifications. The density of 1.06 < d < 1.21 g/ml was isolated by sequential ultracentrifugation, dialyzed against a 1 mM EDTA solution, pH 7.4, brought to 6 M guanidine hydrochloride, and incubated for 3 h at 37 °C. Hereafter, it was dialyzed again against 1 mM EDTA, pH 7.4, readjusted to a density of d = 1.21 g/ml by adding solid KBr, and centrifuged at 50,000 rpm for 3 h in a 60 Ti rotor (Beckman Instruments). After the spin, the top 5-ml fraction was used to isolate apo-A-II and the bottom 10-m1 fraction to purify apo-A-I by chromatography on a DEAE-Sephacel column.

Apolipoprotein A-IV was isolated from human chylomycin plasma by effusion chromatography and subsequent preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described by Utermann and Beisiegel (3). Apolipoprotein A-IV was also isolated from the d > 1.21 g/ml density fraction of human serum by a modification of the procedure described by Weinberg and Scann (23).

After reconstitution and delipidation of the phospholipid-triglyceride emulsion particles, the protein precipitate was dissolved in a 30 mM Tris-HCl, pH 8.0, buffer containing 6 M urea, chromatographed on a 1.6 × 30 cm DEAE-Sephacel column equilibrated with the same buffer, and eluted with a linear gradient of NaCl from 0 to 125 mM NaCl to separate mainly apo-A-I from apo-A-IV. The apo-A-IV-containing fractions, identified by analytical dodecyl sulfate-polyacrylamide gel electrophoresis, were dialyzed against 5 mM NH₄HCO₃, lyophilized, and subjected to preparative dodecyl sulfate-polyacrylamide gel electrophoresis in a cylindrical gel column in a discontinuous system as outlined by Utermann (24). Fractions containing pure apo-A-IV were again pooled and dialyzed against 0.02 M ethylmorpholine-HCl, pH 8.5, at +4 °C to remove excess dodecyl sulfate, subsequently dialyzed against 5 mM NH₄HCO₃, and lyophilized. The protein was isolated from trace amounts of dodecyl sulfate by precipitation in acetone/ethanol (1:1, v/v) at -20 °C. Apo-A-IV was redissolved in a 10 mM Tris-HCl, pH 7.4, buffer containing 150 mM NaCl and 1 mM EDTA.

**Enzyme Purification**—Lecithin:cholesterol acyltransferase was isolated from fresh human plasma by a combination of ultracentrifugation, affinity chromatography, and ion exchange chromatography. Plasma was adjusted to a density of d = 1.21 g/ml by adding solid KBr, and centrifugation was carried out at 59,000 rpm in a type 60 T rotor using a Beckman model L60 centrifuge (Beckman Instruments). After centrifugation, the lipoprotein-containing fraction of each tube (~8 ml) was aspirated with a syringe, and the adjacent albumin-poor zone (~10 ml) was kept for further lecithin:cholesterol acyltransferase enzyme preparation. This "upper bottom" fraction was dialyzed extensively against 10 mM KCl, 50 mM Tris, pH 7.0, and then passed through a 1.6 × 25 cm column containing Blue Sepharose CL-6B (Pharmacia Fine Chemicals, Uppsala, Sweden) equilibrated with the same buffer. A flow rate of 30 ml/h was maintained. The fractions containing enzyme activity, apo-A-I, and apo-A-IV coeluted with the nonbinding material. The fractions containing the activity were dialyzed against 10 mM Tris-HCl, 1 mM EDTA, pH 7.4, and applied to a 0.9 × 25 cm column containing DEAE-Sephacel (Pharmacia) equilibrated with the same buffer. After application of the activity-containing fractions, the column was washed with 200 ml of buffer, followed by a linear salt gradient (25-200 mM NaCl) in a total volume of 500 ml at a flow rate of 15 ml/h. The elution profile of a DEAE-Sephacel column chromatography is shown in Fig. 1. The fractions containing lecithin:cholesterol acyltransferase were free of immunochemically detectable apo-A-I and apo-A-IV (see Fig. 1), and additionally, no reaction was seen with antibodies against apo-C-I, total apo-C, apo-D, apo-E, and apo-A-II.

The isolation procedure led to a 2500-fold purification of the enzyme. The activity-containing fractions were pooled, and aliquots were kept at -20 °C and used within 4 weeks.

**Lecithin:Cholesterol Acyltransferase Activity Assay**—Enzyme activity was measured by the conversion of [4-¹⁴C]cholesterol to [4-¹⁴C]cholesterol ester in artificial phospholipid/cholesterol/apoprotein substrate. The substrate was prepared by two different procedures (A and B). To prepare substrate A (lecithin/cholesterol suspension), 1.9 µg of unlabeled cholesterol, 0.7 µg of [4-¹⁴C]cholesterol (0.1 µCi), and 40 µg of phosphatidylcholine/assay tube dissolved in 50 µ1 of chloroform were mixed in a test tube and dried under nitrogen. Then 50 µl of 0.05% m-mercaptoethanol/assay tube were added, and the mixture was sonicated at 20 watts for 2 min using a B-12 Sonifier (Branson Sonic Power Company, Danbury, CT). The sonicated lipid suspension was immediately used in the assay, which was carried out in duplicate in 1.5-ml capped micro-test tubes. Into each assay tube, 50 µl of substrate suspension, 50 µl of assay buffer stock solution, 50 µl of various concentrations of apoprotein solutions, and 5.1 µl of 0.06 M m-mercaptoethanol were added (assay buffer stock solution: 20 mM Tris, pH 7.4, 1.3 mM EDTA, 460 mM NaCl, 2% BSA was prepared and heated for 30 min at 56 °C. Aliquots were kept frozen at -20 °C for a maximum of 2 months. The mixture was then vortexed and incubated at 37 °C. After 1 h, 50 µl of purified lecithin:cholesterol acyltransferase preparation were added, and the incubation was continued for another 4 h, except where stated otherwise. The assay was linear during this time (data not shown).

The preparation of lipidic-cholesterol-[4-¹⁴C]cholesterol and apoprotein prepared by the cholate dialysis technique essentially as outlined by Chen and Albers (25) with different molar ratios of phosphatidylcholine/apoprotein as outlined below. It was used essentially in the same way in the lecithin:cholesterol acyltransferase activity assay, except that no apoprotein+ needed to be added and preincubation could be omitted.

In both assay systems, the incubation was terminated by the addition of a chloroform/methanol solution (1:2, v/v), containing 10 µg/ml each of cholesterol and cholesteryl oleate as carriers, and lipids were extracted at room temperature. Cholesterol was separated from cholesteryl ester by thin-layer chromatography on precoated sheets (20 cm × 20 cm) on Silica Gel 60 F₂₅₄ (Merck) with chloroform/methanol/acetone/glacial acetic acid (5:2:1:1, v/v). The radioactivity in the cholesterol and cholesteryl ester regions was determined with a toluene 2,5-diphenyloxazole/1,4-bis[2-(5-phenyloxazolyl)]benzene scintillation mixture in a scintillation counter. Control tubes in each assay containing no lecithin:cholesterol acyltransferase exhibited less than 1% of the maximally obtained cholesterol ester formation by nonenzymatic reaction.

Activation experiments were performed at least twice, some were carried out up to six times with different lecithin:cholesterol acyltransferase and apoprotein preparations. Aging of the enzyme, and variable amounts of enzyme, per assay influenced the absolute amounts of cholesteryl ester formed per assay, but not the relative proportion of cholesteryl ester formation facilitated by various apoproteins tested. The figures show single typical experiments.

**Gel filtration**—Lipid-apoprotein complexes were chromatographed on a Sepharose CL-6B column (1.6 × 60 cm), calibrated with blue dextran 2000, human low density lipoprotein and HDL, BSA, apo-A-I, and dithiothreitol in the Tris-HCl, pH 7.4 (140 mM NaCl, 10 mM Tris, 1 mM EDTA), buffer system.

Density gradient ultracentrifugation was carried out in a SW-41 swinging bucket rotor containing a Beckman model L60 centrifuge (Beckman Instruments) according to the procedure described by Rodgrave et al. (29). Instead of 4 ml of plasma at a density of d = 1.21 g/ml, a lipid apoprotein complex preparation was adjusted to d = 1.21 g/ml in a volume of 4 ml.

**Analytical Methods**—Analytical polyacrylamide gel electrophoresis in 2% dodecyl sulfate was performed in slab gels using the discontinuous buffer system of Neville (27). Acrylamide monomer concentration was 13%. Protein concentrations were determined by the procedure of Lowry et al. (28) using BSA as a standard and individual apoproteins A-I and A-IV additionally by the electromunnoassay procedure of Laurell (29) with some modification (3). 9 × 12-cm glass plates were covered with 1% agarose containing 2.5% polyethylene glycol and 0.02% m-mercaptoethanol/buffer system, pH 8.6. The middle section (5%) of the agarose was removed and replaced by an antibody containing agarose of otherwise identical composition. Samples were diluted with barbital buffer containing 2% Tween X-100, and 5 µl were applied for electrophoresis at 10 V/cm for 4 h. Plates were washed after the run with 0.9% NaCl, dried under a filter paper, and stained with Comassie Brilliant Blue.

**RESULTS**

**Isolation of Lecithin:Cholesterol Acyltransferase**—Lecithin:cholesterol acyltransferase was isolated by a combination of ultracentrifugation, affinity chromatography on Blue Sepharose, and DEAE-Sephacel chromatography as described under "Experimental Procedures." The enzyme that eluted from the DEAE column was free of detectable apo A-I and apo-A-IV (see Fig. 1) and was virtually inactive in the absence of a
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**FIG. 1.** Isolation of lecithin:cholesterol acyltransferase by DEAE-Sephacel column chromatography. Lecithin:cholesterol acyltransferase was prepurified from human plasma by ultracentrifugation and affinity chromatography on Blue Sepharose as described under "Experimental Procedures." After affinity chromatography, lecithin:cholesterol acyltransferase activity-containing fractions were applied to a DEAE-Sephacel column (0.9 x 25 cm) and eluted with a linear NaCl gradient (---). Apo-A-I (O--O) and apo-A-IV (O--O) were well separated from lecithin:cholesterol acyltransferase enzyme activity (O--O). Absorbance 280 nm.

**FIG. 2.** Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of apolipoproteins. Lipoprotein-depleted serum was incubated with a phospholipid/triglyceride emulsion in the presence of 4 M NaCl, and apoproteins complexed with the lipid emulsion were isolated by ultracentrifugation (lane 1). Apo-A-IV was isolated from the delipidized apoprotein mixture by DEAE-chromatography and preparative SDS-polyacrylamide gel electrophoresis (lane 2). Apolipoprotein E standard (lane 3). HSA, heavy strand.

protein cofactor when tested with lecithin:cholesterol complexes as substrate (see below).

**Isolation of Apo-A-IV**—Apolipoprotein A-IV was isolated from two sources, from lipoprotein-depleted human serum, as described by Weinberg and Scanu (23), and from chylomicrons in chylous pleura effusion of a patient with chylothorax as described by Utermann and Beisiegel (3). These preparations still contained immunochemically detectable apolipoprotein A-I. Therefore, we included DEAE-Sephacel chromatography as an additional purification step. This resulted in apo-A-IV preparations that were free from other apolipoproteins, including apo-A-I and apo-C-I as judged by electroimmunodiffusion and SDS-polyacrylamide gel electrophoresis. Upon SDS-polyacrylamide gel electrophoresis, apo-A-IV migrated as a single band of $M_r \sim 46,000$ (Fig. 2). Unless otherwise stated, apo-A-IV from lipoprotein-depleted serum was used in the experiments described below.

**Activation of Lecithin:Cholesterol Acyltransferase by Apo-A-IV**—Increasing amounts of apolipoproteins A-IV, A-I, or A-II were added to sonicated suspensions of egg yolk phosphatidylcholine/cholesterol/$[^{14}C]$cholesterol and the apoprotein-lipid complexes thus formed were incubated in the presence of purified lecithin:cholesterol acyltransferase enzyme (method A). As previously described by several investigators (21, 25, 30-33), the cholesteryl ester formation by lecithin:cholesterol acyltransferase was facilitated in the presence of apo-A-I. Maximal activation was obtained by 1 pg of apo-A-I under our assay conditions. No cholesteryl ester formation was observed when either the activator protein or the enzyme was omitted from the assay mixture or when apo-A-I was present (Fig. 3). Addition of apo-A-IV, however, significantly stimulated the cholesteryl ester formation by lecithin:cholesterol acyltransferase. Using egg yolk lecithin as acyl donor, apo-A-IV exhibited about 12-20% of the activator activity of apo-A-I (Fig. 3).

To confirm this result, we prepared apoprotein-lipid complexes by the detergent dialysis procedure (method B), using a lipid/apoprotein ratio (PC/apoprotein, ~500:1) that had resulted in maximal activation under the conditions of method A (see above). Apolipoproteins A-I and A-IV both were much more active in facilitating the reaction when incorporated into apoprotein-lipid complexes than when added to the lipid suspension preformed by sonication (Table I). This effect was even more pronounced with apo-A-IV than with apo-A-I (Table I). Cholesteryl ester formation with apo-A-IV-lipid complexes was about 35-45% of that seen with apo-A-I-lipid complexes.
...where increasing amounts of apoproteins were added to the incubation was continued for another one unsaturated fatty acid from all tested PCs with one saturated and one unsaturated fatty acid esterified with one saturated and one unsaturated fatty acid.

Apo-A-IV was able to activate the lecithin:cholesterol acyltransferase reaction, but apo-A-I was always a more potent activator of the enzyme with these substrates. It was not important whether the saturated or unsaturated fatty acid was located in the sn-2-position of the phospholipid. With both L-α-1-palmitoyl-2-oleoylphosphatidylcholine and L-α-1-oleoyl-2-palmitoylphosphatidylcholine, apo-A-I and apo-A-IV exhibited similar cofactor activities (Fig. 4).

(c) Third, lipid substrate complexes were prepared with phospholipids that were esterified with two saturated fatty acids (dipalmitoyl-, dimyristoyl-, and didecanoylphosphatidylcholine). Complexes of either of these phospholipids with cholesterol were better substrates for the lecithin:cholesterol acyltransferase-mediated esterification reaction in the presence of apo-A-IV than in the presence of apo-A-I (Fig. 4).

Hence, under the conditions of method A, it depended on the nature of the phospholipids in the substrate complex whether apo-A-I or apo-A-IV were more potent activators for the enzyme reaction. Apo-A-I exhibited a better activity with apo-A-IV (left), L-α-dipalmitoyl-PC (middle), and L-α-didecanoyl-PC (right), apo-A-IV was always a better activator than apo-A-I. Assay conditions are the same as outlined in Fig. 3, apo-A-I, apo-A-IV, A-I, and apo-A-IV.

**TABLE I**

Formation of cholesterol ester by lecithin:cholesterol acyltransferase under different substrate conditions

| Substrates were prepared at equal molar ratios of egg yolk lecithin/cholesterol/apoprotein by method A or method B. | Cholesterolester formed per assay (μg protein) |
| --- | --- |
| Apoprotein | Method A | Method B |
| A-I | 262 | 570 |
| A-IV | 34 | 217 |
| A-II | 5 | 20 |

**FIG. 3.** Activation of the lecithin:cholesterol acyltransferase reaction by apolipoproteins under different phospholipid substrate conditions. Increasing amounts of apolipoprotein were added to sonicated suspensions of phospholipid/cholesterol/[14C]cholesterol in Tris buffer containing BSA and β-mercaptoethanol (method A). The mixtures were incubated at 37°C for 3 h prior to the addition of purified lecithin:cholesterol acyltransferase enzyme, and the incubation was continued for another 4 h. Cholesteryl ester formation was determined as described under "Experimental Procedures". Left panel, activation of lecithin:cholesterol acyltransferase by apo-A-I (●), apo-A-II (▲), and apo-A-IV (□) with egg yolk phosphatidylcholine as acyl donor. Right panel, activation of lecithin:cholesterol acyltransferase by apo-A-I (○), apo-A-II (●), and apo-A-IV (▲) with dioleoyl-PC and by apo-A-I (●) with egg lecithin as acyl donors.

**FIG. 4.** Activation of lecithin:cholesterol acyltransferase by apo-A-I and apo-A-IV with different PC substrates. The upper panel shows the results with phosphatidylincholines substituted with one saturated and one unsaturated fatty acid residue: with L-α-palmitoyl-2-oleoyl-PC (left), L-α-oleoyl-2-palmitoyl-PC (middle), and L-α-palmitoyl-2-linoleoyl-PC (right), apo-A-I was a better activator than apo-A-IV. The lower panel shows the results obtained with phosphatidylincholines esterified with two saturated fatty acids. With L-α-dimyristoyl-PC (left), L-α-dipalmitoyl-PC (middle), and L-α-didecanoyl-PC (right), apo-A-IV was always a better activator than apo-A-I. Assay conditions are the same as outlined in Fig. 3, apo-A-I, apo-A-IV, A-I, and apo-A-IV.
Apo-A-II preparation did not contain apo-A-I or apo-A-IV.

**Substrates Prepared by Detergent Dialysis Procedure**—To further confirm that apo-A-IV exhibits cofactor activity in the lecithin:cholesterol acyltransferase reaction, we prepared DMPC-cholesterol-[1,4-C]cholesterol-apoprotein complexes by the detergent dialysis procedure (method B) using a phospholipid/protein molar ratio of 150:1. This ratio is known to result in good artificial substrate in the case of apo-A-I (33). The DMPC-cholesterol-[1,4-C]cholesterol-apoprotein complexes containing either apo-A-I or apo-A-IV were characterized by gel permeation chromatography and by density gradient ultracentrifugation. Upon gel permeation chromatography on Sepharose CL-6B, complexes containing apo-A-IV appeared to be homogenous and slightly larger than apo-A-I-containing particles (elution volume 79.3 versus 85.3 ml, see Fig. 5).

Upon density gradient ultracentrifugation, apo-A-I- and apo-A-IV-containing complexes both appeared homogenous and had a density of about 1.1 g/ml. Cholesterol, phospholipid, and apoprotein had essentially the same distribution in the gradient (Fig. 6). When the DMPC-cholesterol-apoprotein complexes were tested as substrates for the lecithin:cholesterol acyltransferase reaction, we obtained in principle the same results as with method A. Complexes containing apo-A-IV were better substrates for the enzyme than those containing apo-A-I (see Table II). Together, these data indicate that apo-A-IV forms defined particles with DMPC and cholesterol in a similar way, as apo-A-I and that these particles are good substrates for the lecithin:cholesterol acyltransferase reaction.

**Cholesteryl Ester Formation in the Absence of Activator Proteins**—In the experiments with DPPC, DMPC, and DDPC, we observed a substantial amount of cholesteryl ester formation even in the absence of activator proteins (see "Experimental Procedures"). None of the other phospholipids tested exhibited this effect (see Fig. 3 and Fig. 4, upper panel). We therefore started to investigate this phenomenon more systematically. Phospholipid-cholesterol-[1,4-C]cholesterol complexes were prepared by sonication (method A) with each of the phosphatidylcholines containing two saturated fatty acids (DDPC, DMPC, DPPC, DSPC) and for comparison with egg lecithin. The complexes were incubated with a constant amount of purified enzyme but in the absence of activator proteins for various time periods. With DMPC, DDPC, and DPPC, time-dependent linear formation of cholesteryl ester was observed. Cholesteryl ester formation was most pronounced with DMPC and DPPC (Fig. 7). No cholesteryl ester formation was seen with complexes containing lecithin or DSPC. The results of this experiment and the immunochemical analysis of the enzyme preparation make it unlikely that the observed formation of cholesteryl ester is due to the presence of some activator protein in the lecithin:cholesterol acyltransferase preparation. Essentially, the same results were obtained when complexes were formed by the detergent dialysis procedure (method B, see Table II).

**DISCUSSION**

It is generally accepted that the plasma enzyme lecithin:cholesterol acyltransferase requires a protein activator...
Tris buffer containing BSA, & mercaptoethanol, and purified lecithin:cholesterol acyltransferase and different phospholipid substrates were added, and incubation was carried out by pipetting a sonicated suspension of the lipid and addition of apoprotein activator apoproteins. With egg yolk lecithin (A) or L-α-distearylphosphatidylcholine (Δ), substantial amounts of cholesteryl ester were formed in the absence of activator apoprotein. No cholesteryl ester formation was seen with egg yolk lecithin (●) or L-α-dioleoylphosphatidylcholine (○).

originally found to be the major protein component of high density lipoproteins, apolipoprotein A-I (21). Later, another apoprotein, apo-C-I from human plasma (30), and a synthetic peptide analogous to apo-C-I (34) were also shown to exhibit substantial activating capacity for lecithin:cholesterol acyltransferase. The results of the present research clearly demonstrate that apolipoprotein A-IV is a further potent activator of the plasma enzyme lecithin:cholesterol acyltransferase in vitro.

In our work, the activating capacity of apo-A-IV for the lecithin:cholesterol acyltransferase reaction was always investigated in comparison to the “standard” activator apo-A-I under different substrate conditions. This revealed a broad range of “activator activities” for both apoproteins. The transacylation reaction depended on both the apoprotein and the phospholipid component in the artificial substrate complexes. With phospholipid substrates that contained two saturated fatty acid residues, apo-A-IV was more effective in facilitating the enzyme reaction than apo-A-I. The opposite was true for DOPC and for phospholipids that were esterified with one saturated and one unsaturated fatty acid, no matter which position was occupied by the unsaturated or saturated fatty acid, respectively. However, also with these phospholipid substrates, significant amounts of cholesteryl ester were formed with apo-A-IV as activator protein.

The differences in reactivity of apoprotein-lipid complexes prepared by sonication of the lipid and addition of apoprotein may in part be explained by the fact that individual phospholipids react differently with various apoproteins in some cases, giving rise spontaneously to discoidal particles (45–47). Therefore, we also investigated the reaction of complexes prepared by the cholate dialysis procedure. In agreement with other authors (25, 32, 33), apoprotein-lipid complexes prepared in the presence of cholate were better substrates for the lecithin:cholesterol acyltransferase reaction than substrates prepared by the addition of apoprotein to a sonicated dispersion of lipids. Products of the cholate preparation are micellar, disc-like particles more reactive than vesicles of equal composition. The increase in reactivity was observed with egg yolk lecithin (Table I), DMPC, and DOPC (data not shown).

Recent studies by De Lamatre et al. (35) have provided evidence that the apo-A-IV distribution in rat plasma can be influenced by the lecithin:cholesterol acyltransferase reaction. They have shown that apo-A-IV moves from the lipoprotein-free fraction to HDL in association with the lecithin:cholesterol acyltransferase-mediated cholesterol esterification. Together with our findings, this indicates that apo-A-IV is involved in the lecithin:cholesterol acyltransferase reaction.

The precise mechanism by which the activator proteins facilitate the lecithin:cholesterol acyltransferase reaction is not completely understood. Jonas et al. (44) recently concluded from their studies that enzyme activation occurs upon binding to complexes via apoproteins which depend on apolipoprotein structure that is not exclusively determined by the α-helical content. Others concluded from their investigations that there is no strong interaction between apo-A-I and the enzyme (31). From studies of a series of native and synthetic fragments of apo-A-I and of nonrepeptide-related, it was concluded that the interaction of the cofactor protein with lipids is of prime importance for the lecithin:cholesterol acyltransferase-activating capacity of the apoprotein (for review see Ref. 36). The common feature of all peptides that facilitates the lecithin:cholesterol acyltransferase reaction is their ability to form amphiphilic helices with a hydrophobic and a hydrophilic face that enable them to interact with lipids.

The lecithin:cholesterol acyltransferase enzyme-stimulating activity of apo-A-I is believed to be a function of its multiple 22-residue amphipathic segments (37, 38, 39). Our finding that apo-A-IV facilitates the lecithin:cholesterol acyltransferase reaction predicts that this protein has structural similarities to apo-A-I and, in particular, that it has a similar amphipathic moment. Recent studies by Boguski and coworkers (40) indeed have provided evidence for this concept. These authors have shown that rat apolipoprotein A-IV contains 13 tandem repetitions of a 22-amino acid segment with amphipathic helical potential and have suggested that apo-A-IV and apo-A-I have evolved from divergence of a common ancestral gene. They have postulated that apo-A-IV is an effective lecithin:cholesterol acyltransferase activator (40). Their prediction is confirmed here by direct experimental evidence. Apolipoprotein A-IV not only forms stable complexes with DMPC/cholesterol mixtures but, moreover, greatly enhances the activity of lecithin:cholesterol acyltransferase with this and other lipid substrates.

Yokoyama and co-workers (39) have concluded from their studies employing model docosapeptides designed to be prototypic amphipathic α-helices that the presence of a large enough amphipathic structure is sufficient for lecithin:cholesterol acyltransferase activation. However, there are several observations that argue for more specific conformational requirements for an effective lecithin:cholesterol acyltransferase activator protein. One is that other apoproteins that also are believed to contain amphipathic segments as apo-C-II, apo-C-IIIα, -C-IIIβ, and apo-A-II are poor activators of the reaction (30, 41, 44), as shown here for apo-A-II. The relative reaction rate of apo-A-II versus apo-A-I in egg yolk-cholesterol complexes in our experiments is in agreement with the one reported by Jonas et al. (44). Apo-A-IV on the other hand exceeds the highest relative reaction rate reported for apo-C-I by far (36% versus 12.1% reported by Jonas et al. (44)). Among all apoproteins tested so far for their relative reaction rate versus apo-A-I with egg yolk lecithin as acyl donor, apo-A-IV is most reactive. This demonstrates that the lipid-binding properties of an apoprotein per se are not suffi-
cient to facilitate the lecithin:cholesterol acyltransferase reaction effectively but rather that the precise spatial orientation of the apoprotein in the boundary lipid layer of lipoproteins is of importance for the activating capacity.

The significance of the maintenance of the secondary structure of apo-A-I for lecithin:cholesterol acyltransferase activation has moreover been illustrated by the study of two mutants of this protein, apo-A-I (Pro 143 → Arg) and apo-A-I (Lys 107 → 0), that are defective in their ability to activate the enzyme (42, 43). The type and position of the amino acid interchange in apo-A-I (Pro 143 → Arg) and the Lys deletion in position 107 probably disrupt the secondary and/or tertiary structure of apo-A-I (42, 43).

An interesting but unexplained finding is our observation that complexes of phospholipids containing two saturated fatty acid residues can serve as substrates for lecithin:cholesterol acyltransferase even in the absence of an activator protein. In such complexes there may be "gaps" in the enzyme to interact with the hydrocarbon chains without the helper function of an apoprotein. With the same phospholipid substrates, apo-A-IV is a better activator of the enzyme than apo-A-I. Whether this in vitro activating capacity of apo-A-IV is also significant in vivo remains to be established.

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