Structural studies of *Manduca sexta* lipid transfer particle with apolipoprotein-specific antibodies

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Abstract Studies have been conducted to characterize structural and functional properties of *Manduca sexta* lipid transfer particle (LTP). LTP is a high molecular weight complex of three apolipoproteins and lipid that facilitates the transfer of lipids between lipoproteins and tissues and among lipoproteins in insect hemolymph. Rabbit polyclonal antibodies were raised against each of the three LTP apolipoproteins isolated by preparative electrophoresis. Immunoblot experiments demonstrated that they are apolipoprotein-specific and that LTP apolipoproteins are immunologically distinct polypeptides. Antibody-capture enzyme-linked immunosorbent assay characterization of apolipoprotein-specific IgG demonstrated that each of the three antibodies recognizes native LTP and provided information on the apparent affinity of the antibodies for LTP. Apolipoprotein-specific IgG were then compared in lipid transfer assays to examine the effect of antibody binding on LTP-mediated lipid transfer. Although each of the antibodies inhibited transfer activity, anti-apoLTP-II was capable of nearly abolishing activity at low IgG concentrations (< 26.7 μg IgG/μg LTP). In contrast, anti-apoLTP-I and anti-apoLTP-III IgG inhibited LTP activity only at much higher concentrations (> 133.3 μg IgG/μg LTP). These results indicate that apoLTP-II is a catalytically important apolipoprotein. In immunoprecipitation experiments, using ^{125}I-labeled LTP, anti-holoLTP, anti-apoLTP-I, and anti-apoLTP-II were each able to immunoprecipitate all three LTP apolipoproteins while anti-apoLTP-III was not. When immunoprecipitations were carried out in the presence of the nondenaturing detergent, Nonidet P-40, however, anti-apoLTP-I and anti-apoLTP-II IgG were able to immunoprecipitate only apoLTP-I and -II while apoLTP-III was not immunoprecipitated. Only apoLTP-III was immunoprecipitated by anti-apoLTP-III under these conditions. As expected, anti-holoLTP immunoprecipitated all three substituents in the presence of Nonidet P-40. These data suggest that apoLTP-III can dissociate from the LTP complex upon treatment with nondenaturing detergent while apoLTP-I and -II remain associated. The results also suggest that apoLTP-III interacts with the lipid component of the LTP complex. Solubilization with detergent appears to disrupt this interaction, allowing the dissociation of apoLTP-III.—**Blacklock, B. J., and R. O. Ryan.** Structural studies of Manduca sexta lipid transfer particle with apolipoprotein-specific antibodies. *J. Lipid Res.* 1995. **36:** 108–116.

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Vertebrate and invertebrate animals alike solve the dilemma of transporting water-insoluble lipids through their aqueous circulatory systems by assembling lipoprotein complexes. These particles sequester hydrophobic molecules from the environment within a shell of amphipathic protein and lipid. Key aspects of lipid transport include the initial recruitment of lipid by nascent lipoproteins and the interconversion of lipoproteins in the plasma compartment. Lipid transfer catalysts, such as the cholesteryl ester transfer protein (CETP; 1), microsomal triacylglycerol transfer protein (MTP; 2, 3), and insect hemolymph lipid transfer particle (LTP; for review see reference 4) have been shown to play a role in both these processes. LTP has been purified from three insect species (5–7) and identified in a fourth (8). It is a high molecular weight ($\sim 1 \times 10^6$ Da) complex comprised of three apolipoproteins and noncovalently associated lipid. In *M. sexta,* LTP apolipoproteins (apoLTP-I; 320,000 Da, apoLTP-II; 85,000 Da, and apoLTP-III; 55,000 Da) exist in a stoichiometry of 2 apoLTP-I: 2 apoLTP-II: 1 or 2 apoLTP-III (9–11). Electron microscopic studies of LTP have demonstrated an unusual, highly asymmetric morphology with a quasi-spherical head and a hinged tail region (7, 11). LTP has been shown to play a role in the transfer of diacylglycerol (DAG) from fat body to lipophorin, the major insect hemolymph lipoprotein (12). LTP is also thought to be involved in the interconversion of

Abbreviations: apoLTP-I, II, III, lipid transfer particle apolipoprotein-I, -II, -III; ABTS, 2,2'-azino-di-[3-ethylbenzthiazoline] sulfonate; DAG, diacylglycerol; ELISA, enzyme-linked immunosorbent assay; HDLp, high density lipophorin; IgG, immunoglobulin class G; LDL, human low density lipoprotein; MTP, microsomal triacylglycerol transfer protein; PBS, phosphate-buffered saline; PVDF, polyvinylidine difluoride; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; TTBS, Tris-buffered saline/1.0% Tween-20.

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lipophorin subspecies within the hemolymph (13, 14) and in the uptake of lipid by developing oocytes (15). In vitro lipid transfer experiments have shown that LTP, in contrast to other known lipid transfer proteins, can facilitate net vectorial lipid transfer among donor and acceptor lipoproteins (16, 17) and induce dramatic changes in substrate particle composition and structure (17–19). LTP uses a carrier-mediated mechanism in lipid transfer (20) wherein its lipid component is in dynamic equilibrium with that of substrate particles (9).

The large size, unusual morphology, presence of lipid and three apoproteins, and lipid transfer ability of LTP poses intriguing questions about the structure, function, and activity relationships of the particle and makes LTP unique among plasma lipid transfer catalysts. The mammalian plasma lipid transfer catalysts, CETP and phospholipid transfer protein, are responsible for the exchange of triacylglycerol, cholesteryl ester, and phospholipids between lipoproteins within the plasma (1). Both proteins are monomeric and have approximate M_r of 74,000 (1) and 70,000 (21), respectively. Generally, intracellular lipid transfer catalysts are small, monomeric polypeptides (22) with the exception of the liver and intestinal MTP (23). This has led to speculation that the smaller LTP apolipoproteins are proteolytic products of apoLTP-I or that copurification of multiple proteins is occurring. The apolipoprotein(s) responsible for the lipid transfer ability of LTP are not known. Neither the physiological and biochemical importance of the unique morphology nor the spatial relationships of the apolipoproteins within the particle have been defined. Previous work has suggested that apoLTP-III is buried within the particle and apoLTP-I and apoLTP-II are exposed (11) but examination by a different experimental means is required. In the present study, polyclonal antibodies against each of the individual apolipoproteins have been prepared and used to probe the particle. The results obtained provide new information on the immunorelationship between apolipoproteins, the identity of a catalytically important apolipoprotein, and the structural organization of the particle.

EXPERIMENTAL PROCEDURES

Isolation of LTP, lipophorin, and LDL

Insects were maintained in a continuous laboratory colony reared on artificial diet as described elsewhere (24). LTP was isolated from hemolymph of 7-day-old, fifth instar, prepupal *Manduca sexta* according to Ryan, Howe, and Scraba (11). Briefly, hemolymph was subjected to an initial density gradient ultracentrifugation to remove high density lipophorin (HDLp; 25). The infranatant fractions containing LTP were pooled, concentrated, and chromatographed on a column of Bio-Gel A1.5. LTP-containing fractions were subsequently applied to DEAE Bio-Gel and eluted with 50 mM sodium phosphate, pH 7.5, containing 300 mM NaCl. The column eluant was then subjected to a second density gradient ultracentrifugation at higher salt concentration, affording the flotation of LTP. Human LDL was isolated from freshly collected plasma between density limits of 1.006 and 1.063 g/ml by sequential density ultracentrifugation. Protein concentrations were determined by the bicinchoninic acid assay method (Pierce Chemical Co.) using bovine serum albumin as a standard.

Preparation of apolipoprotein-specific polyclonal antibodies

Polyclonal anti-sera were raised against apoLTP-I, apoLTP-II, and apoLTP-III by repeated intramuscular injection of isolated apolipoproteins into rabbits. Apolipoproteins were isolated by SDS-PAGE (4–15% linear acrylamide gradient) of LTP (1 mg) by the method of Laemmli (26) using a preparative (3 mm) gel. In the case of apoLTP-I and apoLTP-III, gel slices were excised from the Coomassie Blue-stained gel, lyophilized, macerated, and injected with Freund's adjuvant system (Sigma). This procedure did not produce antibodies against apoLTP-II even after repeated attempts. Anti-apoLTP-II serum was obtained by injection of apoLTP-II, eluted from a macerated gel slice by end-over-end shaking with elution buffer (50 mM Tris-HCl, 150 mM NaCl, 0.1% SDS, pH 8.0), into a rabbit with Ribi's adjuvant (Ribi ImmunoChem Research Inc.). Non-immune serum was obtained by collecting blood from rabbits prior to injection of antigen. Polyclonal antibodies against native LTP were produced by repeated injection of purified LTP (1 mg) with Freund's adjuvant. Anti-larval serum protein serum prepared previously (27) was used as an irrelevant antibody. Immunoglobulin G (IgG) were purified from anti and non-immune sera with Protein-A agarose (Sigma) as described by Harlow and Lane (28). Briefly, the pH of the sera was adjusted to pH 8 by the addition of 1/10 volume of 1.0 M Tris-base, pH 8.0. The sera were applied to Protein-A agarose columns and washed with 10 times column volume each of 100 mM Tris-base, pH 8.0, and 10 mM Tris-base, pH 8.0. IgG were eluted with 100 mM glycine, pH 3.0, concentrated, and dialyzed into phosphate-buffered saline (PBS; 100 mM sodium phosphate/150 mM NaCl/25 mM EDTA, pH 7.0).

Immunoblotting of purified LTP with apolipoprotein-specific antibodies

Purified LTP (0.2 μg) was probed with anti and non-immune sera after transfer from a 4–15% linear acrylamide gradient non-reducing SDS-PAGE gel to polyvinylidene difluoride (PVDF) membrane (Immobilon, Millipore Corp.) under transfer conditions suitable for high

molecular weight proteins (50 mM Tris-base, 380 mM glycine, pH 8.3; 500 mAmps, 3 h, 4°C with cooling coil). Reducing conditions in SDS-PAGE are not required as LTP apolipoproteins are not covalently linked by disulfide bridges (9). The membrane was blocked by gentle shaking for 30 min with 20 mM Tris-base/150 mM NaCl/0.2% Tween-20, pH 7.2 (TTBS) then incubated with non-immune serum (1:2,500; dilution made in TTBS), anti-holoLTP (1:15,000), anti-apoLTP-I (1:10,000), anti-apoLTP-II (1:5,000), or anti-apoLTP-III (1:2,500) for 60 min with gentle shaking. The membranes were then incubated (30 min) with biotinylated goat anti-rabbit IgG (1:5,000 in TTBS; Gibco BRL) followed by streptavidin–horseradish peroxidase (30 min, 1:5,000 in TTBS; Gibco BRL). Extensive washing with TTBS followed each incubation. Detection was achieved by incubation with luminol, a chemiluminescent horseradish peroxidase substrate (ECL, Amersham) and autoradiography.

ELISA characterization of apolipoprotein-specific antibodies

Antibody capture enzyme-linked immunosorbent assays (ELISA) were used to characterize the binding of apolipoprotein-specific antibodies to native LTP. The procedure used was modified from that described by Harlow and Lane (28). Briefly, LTP (1 μg) was bound to the well of Immulon 3 microtiter plates (Dynatech Laboratories) in coating buffer (0.5 M $NaHCO_3$/0.1% NaN_3, pH 9.6) and excess protein binding sites were blocked with 0.5% gelatin/PBS (blocking agent). Apolipoprotein-specific and irrelevant antibodies were diluted in blocking agent to equivalent IgG concentrations as indicated, 200 μl of each were added to wells in triplicate, and incubated at 22°C for 2 h. After extensive washing, horseradish peroxidase-conjugated goat anti-rabbit IgG (Pierce Chemical Co.) diluted in blocking agent (1:3,000) was added, incubated at 22°C for 2 h, and after washing, 2,2'-azino-di-[3-ethylbenzthiazoline] sulfonate (ABTS) substrate solution (Boehringer Mannheim Biochemica) was added to the wells and the absorbance at 405 nm was measured (EAR 340AT Easy Reader microtiter plate reader, SLT-Labinstruments) after 6 min incubation room temperature. Antigen competition antibody capture ELISA experiments were carried out essentially as above except a 100-fold excess LTP was added to specified wells prior to the addition of primary antibody.

Lipid transfer assays

Apolipoprotein-specific antibodies were used to examine the role of the individual LTP apolipoproteins in lipid transfer. An assay of LTP lipid transfer activity that depends upon the induction of sample turbidity upon LTP-mediated vectorial transfer of large amounts of DAG from donor HDLp to acceptor LDL was used. Sample turbidity resulting from LDL particle breakdown and aggregation upon LTP-facilitated transfer of DAG can be quantitated by measuring sample absorbance at 340 nm and is dependent upon LTP and substrate lipoprotein concentration, time, and temperature (17, 25). LTP (0.75 μg, within the linear range of LTP turbidimetric response) was incubated with varying amounts of apolipoprotein-specific or non-immune IgG in a total volume of 80 μl for 16 h at 4°C. LDL (100 μg protein) and HDLp (500 μg protein) were then added to the immune complexes, incubated at 37°C for 1 h, and the absorbance at 340 nm was measured by microtiter plate reader.

Immunoprecipitation experiments

LTP was radioiodinated to a specific activity of 3.3×10^4 cpm/μg with Iodogen beads (Pierce Chemical Co.) according to Markwell (29). Immunoprecipitation reactions were carried out essentially as described by Harlow and Lane (28). Immune complexes were formed by incubation of 0.6 μg ^{125}I-labeled LTP, 0.4 μg LTP as a carrier, and 100 μg non-immune, anti-holoLTP, anti-apoLTP-I, anti-apoLTP-II, or anti-apoLTP-III IgG for 1 h on ice. Protein-A Sepharose (Sigma; 80 ml of 1:1 suspension with PBS) was added and the samples were incubated for 2 h at 4°C with shaking. The tubes were centrifuged, the supernatant was removed, and the beads were thoroughly washed with PBS. Supernatants and beads were treated with sample treatment buffer (8 M urea, 2% SDS, 10% glycerol, 5% β-mercaptoethanol) and boiled 15 min prior to 4–12% acrylamide gradient SDS-PAGE. The gel was dried and autoradiographed. Detergent-containing immunoprecipitation experiments were carried out as described above except the immune complex reactions were carried out with 0.5% NP-40 (Sigma), the Protein-A Sepharose was in a 1:1 suspension with 1% NP-40/PBS, and the beads were washed with 1% Tween-20/PBS.

RESULTS

Immunorelatedness of LTP apolipoproteins

LTP has previously been shown to be immunologically distinct from other hemolymph components (9). In this study, antibodies raised against each of the LTP apolipoproteins were used to examine the immunorelatedness of the apolipoproteins and the specificity of the antibodies. **Figure 1** is an immunoblot of purified LTP probed with anti sera against holoLTP (lane 1), apoLTP-I (lane 2), apoLTP-II (lane 3), apoLTP-III (lane 4), and non-immune serum (lane 5). Anti-holoLTP immunorecognized all three apolipoproteins while each of the antisera raised against a particular apolipoprotein immunoreacted with only the apolipoprotein against which it was raised. There was no cross-reactivity between non-immune serum and LTP.

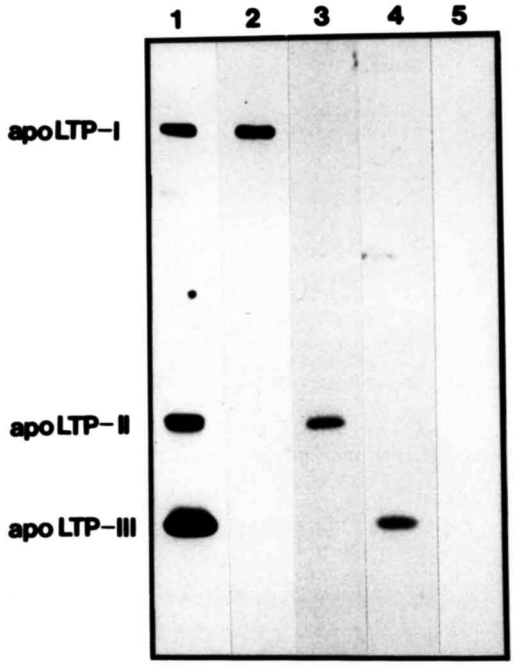

Fig. 1. Immunoblot of purified LTP with apolipoprotein-specific antibodies. Purified LTP (0.2 μg each lane) was run on a 4–12% acrylamide gradient SDS-PAGE gel, transferred to PVDF membrane, and probed with: lane 1, anti-holoLTP (1:15,000 dilution of serum); lane 2, anti-apoLTP-I (1:10,000); lane 3, anti-apoLTP-II (1:5,000); lane 4, anti-apoLTP-III (1:2,500); and non-immune (1:2,500) serum and detected with a biotinylated secondary antibody, a streptavidin-linked horseradish peroxidase and chemiluminescent horseradish peroxidase substrate.

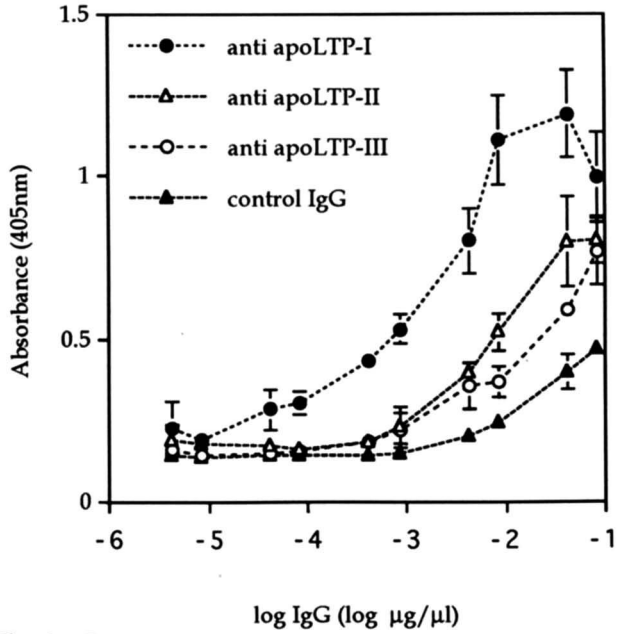

Fig. 2. Determination of apparent affinities of IgG preparations. Purified LTP (1 μg) was bound to wells of Immulon 3 microtiter plates, excess protein binding sites were blocked, and primary antibodies (IgG; anti-apoLTP-I; filled circles, anti-apoLTP-II; open triangles, anti-apoLTP-III; open circles, anti-larval serum protein (control); filled triangles) in varying concentrations were allowed to immunoreact with the bound LTP. The extent of primary antibody binding was determined by detection with a horseradish peroxidase-conjugated goat anti-rabbit IgG and ABTS horseradish peroxidase substrate. The absorbance at 405 nm is plotted versus the log of the IgG concentration. Error bars are the experimental deviation from triplicate determinations.

Characterization of LTP apolipoprotein-specific IgG by ELISA

The ability of the apolipoprotein-specific antibodies to recognize holoLTP was examined by antibody-capture ELISA. This was necessary because the antibodies were raised against denatured apolipoproteins from SDS-PAGE gel slices. **Figure 2** illustrates the IgG concentration dependence of immunoreactivity of each of the apolipoprotein-specific antibody populations to LTP bound to a microtiter plate. These results show the apparent affinity with which each of the antibody populations bind LTP under these conditions. Anti-apoLTP-I binds LTP with the greatest apparent affinity having a half maximum binding value of 2 ng IgG/μl. Anti-apoLTP-II and anti-apoLTP-III bind LTP with lesser apparent affinity yielding the following overall trend in apparent affinity for LTP: anti-apoLTP-I > anti-apoLTP-II > anti-apoLTP-III. There was a slight increase in absorbance at 405 nm at higher concentrations of control IgG (irrelevant anti larval serum protein IgG) due to non-specific reactivity in the assay. In order to verify that the apolipoprotein-specific antibodies recognize LTP in solution, we carried out an antigen competition antibody capture ELISA experiment. **Figure 3** shows the results from the antigen competition experiment in which a 100-fold excess of soluble LTP versus LTP bound to the well was added with the

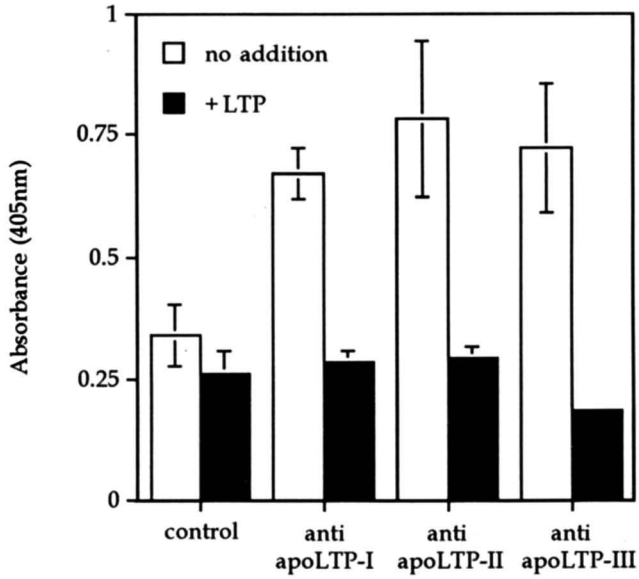

Fig. 3. Competition ELISA with native LTP in solution. Competition ELISA were carried out as described in Fig. 2 except either buffer (no addition, open bars) or 100-fold excess soluble LTP (+ LTP, filled bars) were added with primary antibodies, anti-larval serum protein (16.0 μg), anti-apoLTP-I (0.4 μg), anti-apoLTP-II (1.8 μg), and anti-apoLTP-III (4.0 μg). Absorbance at 405 nm is plotted against the IgG tested. Error bars are the experimental deviation from triplicate determinations.

primary antibody. These results demonstrate that LTP in solution will compete entirely for the binding of all three apolipoprotein-specific antibodies. Together these results demonstrate that each of the apolipoprotein-specific antibody preparations binds native LTP with differing apparent affinities.

Inhibition of LTP-mediated DAG transfer by apolipoprotein-specific antibodies

LTP apolipoprotein-specific antibodies were used to examine the role of individual apo proteins in the catalysis of lipid transfer by LTP. Immune complexes of LTP and anti-apoLTP-I, anti-apoLTP-II, and anti-apoLTP-III were assayed for transfer activity by a turbidimetric assay (25). **Figure 4** shows that each of the apolipoprotein-specific antibody preparations inhibited LTP activity. Anti-apoLTP-II effectively inhibited transfer activity as demonstrated by the lack of sample turbidity at low IgG concentrations (20 μg IgG) while anti-apo-LTP-I and anti-apoLTP-III IgG inhibited activity only when present in large amounts (greater than 100 μg IgG). LTP activity was entirely inhibited by apoLTP-I at 400 μg IgG while anti-apoLTP-III inhibited less than 40% of LTP transfer activity at this concentration. In control experiments, non-immune IgG were unable to inhibit LTP-mediated lipid transfer activity. Incubation of non-immune IgG with substrate lipoproteins, in the absence of LTP, did not result in sample turbidity indicating facilitated DAG transfer did not occur (data not shown). LDL and HDLp did not cross-react with anti holoLTP in a double immunodiffusion experiment (data not shown).

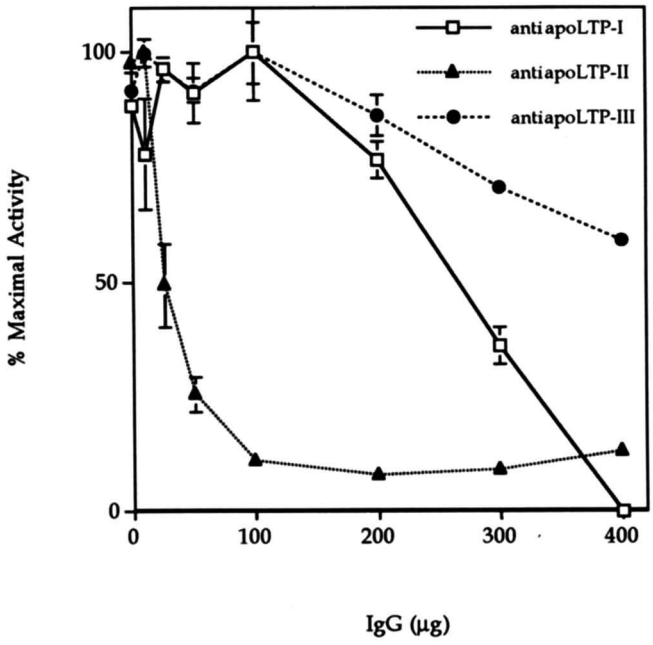

Fig. 4. Effect of apolipoprotein-specific antibodies on LTP activity. LTP (0.75 μg) was incubated with increasing amounts of anti-apoLTP-I (open squares), anti-apoLTP-II (filled triangles), and anti-apoLTP-III (filled circles) IgG to form immune complexes that were assayed for lipid transfer activity by a microtiter turbidimetric assay described in Experimental Procedures. Transfer activity was quantitated as the change in absorbance at 340 nm above control (absence of LTP) and expressed here as % maximal activity. The results presented are representative of experiments carried out three times. Error bars are the experimental deviation from triplicate determinations.

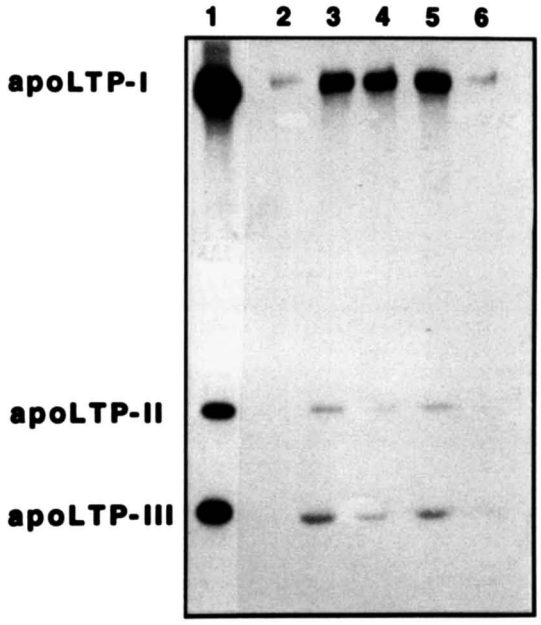

Fig. 5. Immunoprecipitation under native conditions. ^{125}I-labeled LTP (lane 1) was incubated under native conditions with non-immune (lane 2), anti-holoLTP (lane 3), anti-apoLTP-I (lane 4), anti-apoLTP-II (lane 5), and anti-apoLTP-III (lane 6) for 1 h at 4°C. Excess Protein A Sepharose was added and the sample was microfuged to pellet IgG–Protein A Sepharose complexes. The immunoprecipitated material (pellet) was solubilized and electrophoresed on a 4–12% acrylamide gradient SDS-PAGE gel that was dried and autoradiographed.

Immunoprecipitation experiments

The spatial organization and stability of the LTP complex was also examined with apolipoprotein-specific antibodies. LTP was radioiodinated and **Figure 5,** lane 1 demonstrates that each of the three apolipoproteins was radiolabeled. ^{125}I-labeled LTP was used in immunoprecipitation experiments with non-immune, anti-holoLTP, anti-apoLTP-I, anti-apoLTP-II, and anti-LTP-III IgG and Protein-A Sepharose under native conditions. There was a small amount of non-specific immunoprecipitation observed when non-immune IgG was used (Fig. 5, lane 2). Anti-holoLTP, anti-apoLTP-I, and anti-apoLTP-II IgG were all able to immunoprecipitate ^{125}I-labeled LTP (Fig. 5, lanes 3, 4, 5) while anti-apoLTP-III IgG were not (Fig. 5, lane 6).

Immunoprecipitation experiments were also carried out under conditions where non-ionic, non-denaturing detergent was included in the immune complex reaction, precipitation by Protein-A Sepharose, and washing of the beads according to standard protocol. **Figure 6,** panel A

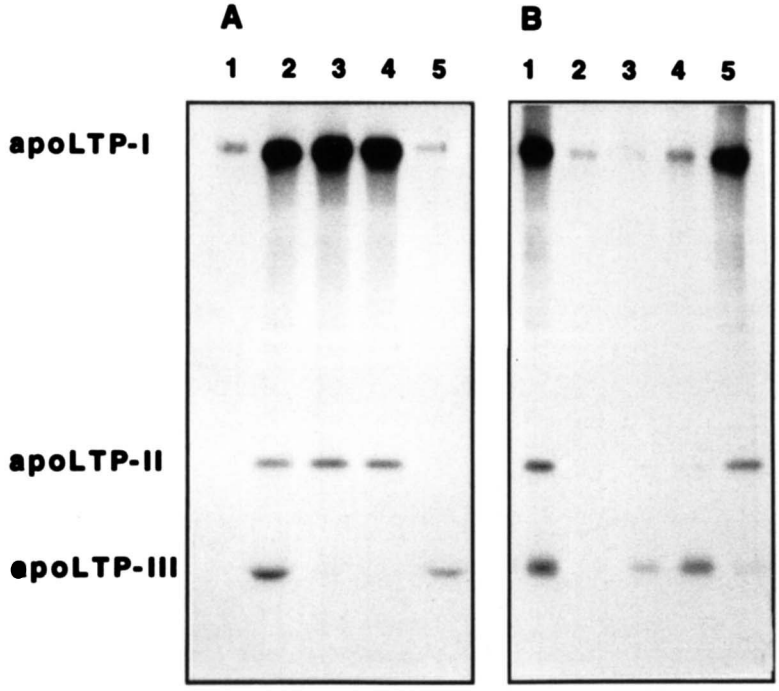

Fig. 6. Immunoprecipitation in the presence of NP-40. ^{125}I-labeled LTP was incubated in the presence of 0.5% NP-40 with non-immune (lane 1), anti-holoLTP (lane 2), anti-apoLTP-I (lane 3), anti-apoLTP-II (lane 4), and anti-apoLTP-III (lane 5). Excess Protein A Sepharose was added and the sample was microfuged to pellet IgG–Protein A Sepharose complexes. Immunoprecipitated (pellets, panel A) and non-immunoprecipitated material (supernatants, panel B) were electrophoresed on a 4–12% gradient SDS-PAGE gel that was dried and autoradiographed.

shows the immunoprecipitated material (pellets) when non-immune (lane 1), anti-holoLTP (lane 2), anti-apoLTP-I (lane 3), anti-apoLTP-II (lane 4), and anti-apoLTP-III (lane 5) IgG were used. Again, there was a small amount of non-specific immunoprecipitation with non-immune IgG. Anti-holoLTP IgG immunoprecipitated all three ^{125}I-labeled LTP apolipoproteins. Anti-apoLTP-I and anti-apoLTP-II, however, both immunoprecipitated only apoLTP-I and apoLTP-II. Panel B, lanes 3 and 4 show that apoLTP-III was not immunoprecipitated and remained in the supernatant of anti-apoLTP-I and anti-apoLTP-II reactions. Anti-apoLTP-III IgG immunoprecipitated only apoLTP-III (Fig. 6, panel A, lane 5) while apoLTP-I and apoLTP-II remained in supernatant (Fig. 6, panel B, lane 5).

DISCUSSION

Polyclonal antibodies specific to each of the LTP apolipoproteins were produced in rabbits upon injection of apolipoproteins separated by preparative SDS-PAGE. Anti-apoLTP-I and anti-apoLTP-III sera were easily raised while it was difficult to obtain anti-apoLTP-II serum, suggesting that apoLTP-II has low inherent immunogenicity. Immunoblot analysis of purified LTP with antisera raised against each apolipoprotein demonstrated that each antiserum immunoreacted with only the apolipoprotein against which it was raised. There was no cross-reactivity between a particular apolipoprotein and antiserum against another apolipoprotein (Fig. 1). These results indicate that LTP apolipoproteins are immunologically distinct polypeptides and that the antibodies are apolipoprotein-specific. The immunological uniqueness of the apolipoproteins confirms that apoLTP-II and -III are not proteolytic products of apoLTP-I. LTP is, therefore, unique among the plasma lipid transfer catalysts in its multimeric composition.

As the apolipoprotein-specific antibodies were raised against denatured protein, it was necessary to demonstrate that they recognized holoLTP and to determine the apparent affinity of the antibodies against holoLTP. Polyclonal antibodies are a mixed population against many different epitopes, so it is not possible to determine a binding constant for each of the anti sera. LTP cannot be resolved into native apolipoproteins because individual apolipoproteins are insoluble in buffer alone, making the determination of a titer for a particular apolipoprotein-specific antibody against the native apolipoprotein impossible. Thus, characterization of apolipoprotein-specific IgG was approached with enzyme-linked immunosorbent assays. Each of the apolipoprotein-specific antibodies was shown to recognize native LTP in antibody-capture ELISA experiments with different apparent affinities (Fig. 2). Anti-apoLTP-I IgG had the highest apparent affinity while anti-apoLTP-II and -III had similar apparent affinities. This is consistent with the composition of the particle as apoLTP-I comprises approximately 60% of the particle mass and would be expected to have a higher apparent affinity for an IgG population. Antigen-competition, antibody-capture ELISA demonstrated that the interaction of the apolipoprotein-specific IgG to LTP bound to

the plate can compete with LTP in solution, further demonstrating that the apolipoprotein-specific antibodies recognize native LTP (Fig. 3).

Numerous studies have demonstrated the catalytic ability of LTP (5–9, 13, 16–19, 30, 31) but the apolipoprotein(s) responsible for lipid transfer have not been determined. We used apolipoprotein-specific antibodies in a lipid transfer inhibition study to examine the catalytically important LTP apolipoprotein(s) (Fig. 4). Anti-apoLTP-II IgG effectively inhibited LTP activity in a turbidimetric assay of DAG transfer (25) at low concentrations of IgG while anti-apoLTP-I and anti-apoLTP-III IgG inhibited activity only at much higher IgG concentrations. The inhibition of transfer activity by anti-apoLTP-II IgG becomes more important when viewed in context with the apparent affinities of the apolipoprotein-specific IgG for native LTP established by ELISA. As anti-apoLTP-I has a greater apparent affinity for native LTP than does anti-apoLTP-II IgG, the results suggest that apoLTP-II is a catalytically important apolipoprotein. Inhibition of transfer activity by high levels of anti-apoLTP-I and anti-apoLTP-III IgG may be due to impaired access of LTP to substrate lipoproteins by the association of anti-apoLTP-I or anti-apoLTP-III IgG with the particle. The results presented here, however, do not entirely rule out a role for apoLTP-I or -III in lipid transfer. Antibodies against epitopes of apoLTP-I or -III involved in transfer may not be present in the antiserum prepared in this study or apoLTP-I or -III may play roles in the interaction of LTP with tissues or lipoproteins in vivo (12, 15).

The apparent key role of apoLTP-II in the transfer activity of LTP suggests similarities between LTP and the mammalian microsomal triacylglycerol transfer protein complex. MTP has two subunits, 88,000 Da and 58,000 Da, the latter being protein disulfide isomerase which is necessary for catalytic activity while the former appears to be the catalytic subunit (2, 23, 32). The similarity in the subunit size of MTP with apoLTP-II and -III and the identification of apoLTP-II as the catalytically important subunit of LTP is interesting. LTP and MTP can be thought of as carrying out similar functions in the biosynthesis and loading of lipoproteins. LTP is involved in the transfer of lipid from its storage site, the fat body, to lipophorin (12) and may have a role in the transfer of dietary lipids into nascent lipophorin particles at the midgut (33). MTP is thought to promote lipid accumulation by nascent apolipoprotein B-containing particles in the endoplasmic reticulum and has been recently found to be the gene defect in abetalipoproteinemia, a disease that is characterized by the absence of apolipoprotein B-containing lipoproteins in the plasma (3, 34).

Apolipoprotein-specific antibodies were used in immunoprecipitation experiments to examine the exposure of apolipoproteins on the surface of the particle and the stability of the complex. Antibodies directed against exposed apolipoproteins would be expected to immunoprecipitate the entire LTP complex while antibodies directed against buried apolipoproteins would be incapable of immunoprecipitating the particle. Results from the characterization of apolipoprotein-specific antibodies by ELISA suggest that each of the antibodies should immunoprecipitate the LTP complex. Radioiodination of LTP resulted in radiolabeling of apoLTP-I, -II, and -III indicating that all three are accessible to radioiodination. ApoLTP-III appeared to be radiolabeled to a greater extent than apoLTP-II suggesting that apoLTP-III has a larger number of tyrosine residues than does apoLTP-II. Immunoprecipitation of ^{125}I-labeled LTP under native conditions demonstrated that apoLTP-I and apoLTP-II are exposed on the surface of the molecule as IgG purified from anti-apoLTP-I and anti-apoLTP-II sera were able to immunoprecipitate intact LTP. Anti-apoLTP-III IgG were not able to immunoprecipitate ^{125}I-labeled LTP indicating that the epitopes against which the apoLTP-III antisera were raised may be inaccessible, although the relatively weak affinity of these antibodies may also be a factor. In general, these results are in agreement with previous studies that demonstrated that apoLTP-III is protected from trypsin degradation while apoLTP-I and -II are not (11). Co-immunoprecipitation of apoLTP-I, -II, -III by anti-apoLTP-I and anti-apoLTP-II provides further evidence that LTP is, indeed, a multimeric complex consisting of three apolipoproteins. The immunoprecipitation results, however, seem to be in contradiction with the ELISA results as anti-apoLTP-III IgG recognizes native LTP almost as well as anti-apoLTP-II IgG. LTP activity inhibition by high concentrations of anti-apoLTP-III also suggests that apoLTP-III is partially exposed but this inhibition may be due to steric hindrance of transfer rather than inhibition by IgG binding to epitopes involved in lipid transfer. These observations may indicate that the antibodies raised against apoLTP-III are not capable of immunoprecipitating apoLTP-III because immunoprecipitation requires a greater affinity to demonstrate a response than ELISA or steric inhibition of transfer. ApoLTP-III epitopes possibly exposed on the surface of LTP may not bind apoLTP-III with a high enough affinity to immunoprecipitate the entire complex.

Interesting results were obtained when the immunoprecipitation was carried out with a mild, non-ionic detergent included in the immune complex and purification of immune complex stages of the experiment. Under these conditions, anti-apoLTP-I and anti-apoLTP-II were both able to immunoprecipitate only apoLTP-I and apoLTP-II and not apoLTP-III which remained in the supernatant of the reaction. Anti-apoLTP-III IgG, however, immunoprecipitated only apoLTP-III and cleared this apolipoprotein from the supernatant of the reaction mixtures. These results indicate that the integrity of the LTP complex is disrupted under these conditions. Mild, non-

ionic detergents, such as Nonidet P-40, are not thought to denature proteins but do solubilize lipid (35). This suggests that apoLTP-III may interact with lipid within the native particle. In the non-detergent-containing immunoprecipitation experiments, anti-apoLTP-III IgG may not access apoLTP-III due to associated lipid and, therefore, would be unable to immunoprecipitate the complex. When non-ionic detergent was included in the immunoprecipitation, the interaction of apoLTP-III with lipid may have been disrupted, exposing apoLTP-III and allowing its dissociation from the particle. Previous studies demonstrated that SDS treatment of LTP rendered apoLTP-III susceptible to trypsin degradation (11) further suggesting that apoLTP-III is protected from the aqueous environment by lipid. ApoLTP-III may not have been immunoprecipitated by anti-apoLTP-I or anti-apoLTP-II in the presence of NP-40 because it had dissociated from the complex. Support for this concept is provided by the observation that anti-apoLTP-III was able to immunoprecipitate only apoLTP-III under these conditions. Thus, it appears that apoLTP-I and apoLTP-II are tightly associated while apoLTP-III can dissociate from the particle upon treatment with non-ionic detergent. Further evidence for the dissociation of apoLTP-III from ^{125}I-labeled LTP upon exposure to NP-40 was obtained by the resolution of apoLTP-III from the complex in NP-40-containing gel permeation chromatography experiments where apoLTP-I and -II remained associated (B. J. Blacklock and R. O. Ryan, unpublished results). In summary, the studies described have resulted in several important findings: *a)* LTP apolipoproteins are immunologically distinct polypeptides; *b)* apoLTP-II is involved in facilitating lipid transfer; *c)* apoLTP-I and apoLTP-II are exposed to the environment and form a more stable complex than that of apoLTP-III with the particle; and *d)* apoLTP-III appears to be associated with lipid.

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