Studies of the morphology and structure of the plasma lipid transfer particle from the tobacco hornworm, *Manduca sexta*

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Abstract Morphological features of *Manduca sexta* plasma lipid transfer particle (LTP) have been investigated by electron microscopy. LTP was found to be an asymmetric particle with two major structural features: a roughly spherical head and an elongated, hinged tail. The hinge occurs approximately at the midpoint of the tail section with the two halves forming angles ranging from 30° to 180°. A molecular mass estimate of 1.4×10^6 daltons based on the dimensions of LTP suggests that multiple copies (two or three) of each of the three LTP apoproteins exist in the native complex. Limited digestion studies of LTP suggest that apoLTP-III is less susceptible to trypsin cleavage than apoLTP-I or -II, and therefore may be less exposed to the aqueous environment. Digestion for 1 h at a 1:50 trypsin–LTP protein ratio did not alter the flotation properties of LTP or its morphological features; thus, although significant proteolysis occurred, the particle retained its overall structure. Transfer activity, on the other hand, was affected by trypsin digestion with 30 ± 14% inhibition of LTP activity occurring upon proteolysis at a 1:50 trypsin–LTP protein ratio. Treatment of LTP with phospholipase A_2 resulted in the conversion of LTP-associated phosphatidylcholine and phosphatidylethanolamine to their corresponding lyso forms. Phospholipase A_2 treatment did not, however, alter the SDS-PAGE profile, transfer activity, flotation pattern, or the microscopic features of LTP. These results suggest that the products of the phospholipase reaction remain associated with the particle. When LTP was pretreated with 0.1% SDS at 25°C, significant morphological perturbation of the head region, loss of catalytic activity, and increased susceptibility to trypsin cleavage were observed. Taken together, our experimental results show that insect plasma LTP is an asymmetric, high molecular weight particle with unique structural and morphological features that are likely to be important in its ability to facilitate the vectorial transfer of lipids. —**Ryan, R. O., A. Howe, and D. G. Scraba.** Studies of the morphology and structure of the plasma lipid transfer particle from the tobacco hornworm, *Manduca sexta*. *J. Lipid Res.* 1990. **31:** 871–879.

Supplementary key word electron microscopy

Hemolymph plasma from the tobacco hornworm, *Manduca sexta*, possesses a catalyst capable of transferring lipid between isolated lipoproteins in vitro (1). Isolation of the entity responsible for this activity has revealed that it is a very high density lipoprotein, which has been termed lipid transfer particle (LTP). LTP is composed of three glycosylated apoproteins (apoLTP–I, $M_r \sim 320{,}000$; apoLTP-II, $M_r = 85{,}000$; apoLTP-III, $M_r = 55{,}000$) and 14% lipid (2, 3). While it is thought that the natural substrate for this catalyst is the diacylglycerol (DAG)-rich major hemolymph lipoprotein, lipophorin (4–6), *Heliothis zea* chromolipoprotein (7), human low density lipoprotein (LDL) (8), egg phosphatidylcholine liposomes (9), as well as human apoA-I-stabilized triacylglycerol/phosphatidylcholine microemulsions (10) are also suitable substrates. The results of studies with different lipid donor/acceptor particle populations has revealed that the structural properties of the donor and/or acceptor have a profound effect on the direction and lipid specificity of transfer as well as being a determinant of whether net transfer or exchange occurs.

Studies using lipophorins containing radiolabeled DAG have shown that the lipid component of LTP is in equilibrium with that of donor and acceptor lipoproteins (3). Treatment of LTP with detergents or ethanol–ether extraction of the lipid component both resulted in loss of catalytic activity. These observations have led to the hypothesis that the lipid component of LTP is involved in the mechanism of catalysis. The specific role of individual LTP apoproteins in the catalysis of lipid transfer is not known. It is possible that one of the LTP apoproteins is the active principle while others are structural components of the complex. The precise arrangement of apoproteins in the LTP complex has not been elucidated.

Abbreviations: LTP, lipid transfer particle; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; LDL, low density lipoprotein; HDLp-L, high density lipophorin-larval; HDLp-W1, high density lipophorin wanderer 1; DAG, diacylglycerol; PBS, phosphate-buffered saline.

Molecular weight estimates of LTP using nondenaturing, pore-limiting polyacrylamide gel electrophoresis and gel permeation chromatography indicate that it is high molecular weight complex of $M_r > 670{,}000$. Thus it has been suggested that more than one copy of each LTP apoprotein may be incorporated into a single particle. In an attempt to obtain further information about the size and shape of LTP, as well as the structural arrangement of apoproteins and lipid in the native complex, we have used electron microscopy and limited digestion with trypsin and phospholipase A_2. The results reveal that LTP is a high molecular weight asymmetric particle whose native structure is retained after these treatments.

MATERIALS AND METHODS

Isolation of LTP

LTP was isolated from the hemolymph of 7-day-old fifth instar prepupal *Manduca sexta* larvae. The insects were reared as described elsewhere (11) and the hemolymph was collected through an incision in the second proleg. Hemolymph was placed directly into 0.1 M sodium phosphate buffer (pH 7.0) containing 0.15 M NaCl, 1 mM EDTA, 1 mM diisopropylphosphorofluoridate, and 5 mM dithiothreitol. Hemolymph was centrifuged at 2,000 *g* to remove hemocytes and the plasma was adjusted to a density of 1.31 g/ml by the addition of solid KBr. The isolation procedure was essentially as described by Ryan, Wells, and Law (2) with the following exceptions. The initial density gradient ultracentrifugation step used an overlay solution of density 1.08 g/ml. This afforded a partial separation of LTP from the bulk of the hemolymph proteins and nearly complete separation from lipophorin which floated to the top of the tube. As a next step, rather than ammonium sulfate precipitation, the tube fractions containing LTP were pooled and concentrated by ultrafiltration prior to Bio-Gel A-1.5 gel permeation chromatography on a column that was equilibrated in 50 mM sodium phosphate, pH 7.5. LTP-containing fractions from this column were then directly applied to a 1.5 × 20 cm DEAE Bio-Gel (Bio-Rad) column equilibrated in 50 mM sodium phosphate, pH 7.5. After washing through unbound proteins the buffer was changed to 50 mM sodium phosphate, pH 7.5, containing 0.2 M NaCl which resulted in elution of LTP activity. Pure LTP was obtained after a second density gradient ultracentrifugation step identical to that described by Ryan et al. (2).

Trypsin digestion

LTP was dialyzed against phosphate-buffered saline (PBS) (0.10 M sodium phosphate, pH 7.0, 0.15 M NaCl, 5 mM EDTA). Samples were placed in 1.5-ml microcentrifuge tubes and an appropriate amount of trypsin (Boehringer) dissolved in PBS was added. After a specified time interval at 25°C a twofold molar excess, with respect to trypsin concentration, of soybean trypsin inhibitor (Boehringer) was added. In some cases trypsinized LTP was subjected to density gradient ultracentrifugation in a Beckman TL-100 ultracentrifuge. Samples were adjusted to 1.31 g/ml by the addition of solid KBr (final volume = 1.5 ml). The sample was then overlayered with 1.5 ml of a solution of KBr (22% w/v) in PBS (density = 1.23 g/ml) and centrifuged at 100,000 rpm for 5 h at 4°C in a 100.3 rotor. Where stated, trypsinization was performed on LTP samples containing 0.1% SDS (w/v) that were preincubated at either 25° or 60°C for 30 min.

Lipid transfer assay

The ability of LTP to catalyze net transfer of DAG was assayed with [^{3}H]DAG-labeled high density lipophorin-larval (HDLp-L) as donor and human LDL as acceptor. The [^{3}H]DAG-HDLp-L was prepared using [9,10-^{3}H]-oleic acid (Amersham, 10 Ci/mmol) as described by Ryan et al. (3) and had a specific activity of 150,000–300,000 cpm/mg protein. Human LDL was purified from fresh plasma at a density between 1.006 and 1.063 g/ml by sequential KBr density gradient ultracentrifugation. The assay method used was similar to that reported by Ryan et al. (3) with the exception that human LDL served as acceptor lipoprotein in the present assays. Briefly, 1 mg protein of each lipoprotein species was incubated at 33°C for 15 min in the presence of 3 μg LTP protein in a final volume of 1.5 ml. HDLp-L and LDL were subsequently reisolated by density gradient ultracentrifugation and the amount of labeled DAG transferred to LDL was determined. In all LTP activity experiments control incubations, lacking LTP, were run in parallel, permitting correction for spontaneous DAG transfer. In all assays the donor, acceptor, and LTP concentrations used were within the linear range of transfer activity response.

Electrophoresis

Samples were analyzed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) according to Laemmli (12). Four to 15% acrylamide gradient slab gels were used with a 2.5% acrylamide stacking gel. Samples were electrophoresed at 30 mA constant current until the tracking dye reached the bottom of the gel. Gels were then stained in a solution of 0.6% Amido Black 10B in methanol–water–acetic acid 50:40:10 (v/v/v).

Phospholipase A_2 Treatment of LTP

Enzymatic digestion was carried out in 1 mM borate buffer, pH 7.5, containing 150 mM NaCl and 10 mM $CaCl_2$. Approximately 70 pmol phospholipid in the LTP holoprotein (equivalent to 0.45 mg LTP) was incubated with 5 units of snake venom phospholipase A_2 (Sigma) in borate buffer in a total volume of 1 ml at room temperature for 120 min. The reaction was terminated by adding excess EDTA with respect to Ca^{2+}.

Lipids were extracted according to Bligh and Dyer (13) and dried under a stream of N_2. Thin-layer chromatography was carried out on glass plates precoated with silica gel 60 (Merck) developed in chloroform–methanol–acetic acid–water 50:30:8:3 (v/v/v/v). After chromatography, lipids were charred with 3% cupric sulfate (w/v)–8% phosphoric acid (v/v) solution and heated at 180°C for 15 min.

Electron microscopy

For conventional negative staining, a 2-μl droplet of LTP, or LTP with high density lipophorin-wanderer 1 (HDLp-W1) (20 μg/ml), in PBS was placed onto a hydrophilic carbon film (prepared by glow discharge), which was supported by a parlodion film on a 300-mesh copper grid. After 1 min the droplet was removed and the carbon surface was washed with 5 droplets of reticulocyte standard buffer (10 mM NaCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl; pH 7.0). The adherent particles were negatively stained by applying 3 droplets of an aqueous solution of sodium phosphotungstate (2%, w/v; pH 7.0) or uranyl acetate (1.5%, w/v; pH 4.4). HDLp-W1 was isolated from hemolymph of prepupal animals on the seventh day following the molt to the fifth larval instar as described elsewhere (11).

Metal replicas of LTPs were obtained using essentially the procedure described by Flicker, Walliman, and Vibert (14). The particles were diluted into a solution of 250 mM ammonium acetate in 50% glycerol and sprayed from a glass microdroplet sprayer onto freshly cleaved mica. The droplets were allowed to dry in a Balzers BA511M apparatus under a vacuum of 10-6 Torr for 1 h. The particles were shadowed, while rotating at 80 rpm, with 1.8 nm of 95% Pt - 5% C evaporated by electron bombardment. The angle of shadowing was 8°, and the amount of Pt-C evaporated was measured with an oscillating quartz crystal. The resulting replica was coated from above with 15 nm of evaporated carbon, then removed from the Balzers apparatus, floated off the mica onto distilled water, and picked up from above with a copper grid.

Negatively stained preparations were examined and photographed in a Philips EM420 electron microscope operated at 100 KV. Off-axis focusing using the low-dose module was employed to minimize specimen damage. The microscope had been calibrated using catalase crystals as a magnification standard (15). Micrographs were taken at an instrumental magnification (calibrated) of 74,400 and recorded on Kodak SO-163 film (3 1/4" × 4" sheets). Development was in D19 (1:2) for 4 min.

The Pt-C replicas were also examined in the EM 420, but for improved contrast photographs were recorded on 35 mm PanX film from a high resolution photomonitor attached to the STEM unit of the microscope. The bright-field/dark-field imaging mode was selected for image formation.

RESULTS

Morphological studies

Molecular weight estimates for insect plasma LTP using nondenaturing polyacrylamide gel electorphoresis and gel permeation chromatography have indicated a high molecular weight for the complex (2, 3). The precise apoprotein stoichiometry as well as the structural arrangement of apoproteins and lipid in the complex are unknown. Given the apparent large size of LTP we chose to examine its morphological features by electron microscopy. Micrographs of negatively stained preparations (**Fig. 1**) showed that LTP has an asymmetric shape. The complex appears to have two distinct features: a roughly spherical head coupled to an elongated tail. The appearance of a variety of conformations of the tail suggest that it has a central hinge. Measurements based on the size and shape of the complex in samples stained with 2% sodium phosphotungstate or 1.5% uranyl acetate revealed that LTP has a high molecular weight, consistent with earlier results obtained by electrophoresis in nondenaturing gels (3) and molecular sieve chromatography (2). A molecular weight estimate was obtained in the following manner. The head region was assumed to be a sphere with r = 6.5 nm, and the tail section (in the 180° conformation) was considered to have a tapered cylindrical shape 38 nm in length with an average r = 2.5 nm. These dimensions represent a molecular volume of 19×10^{-19} cm^3. Combining this value with the previously determined density for LTP = 1.23 g/ml (2) gives a value for the particle weight of 1.4×10^6.

Since it possesses the ability to transfer lipid, LTP must interact with substrate lipoproteins. Therefore we combined LTP and insect plasma high density lipophorin (11) and examined the mixture by electron microscopy (Fig. 1C). Clearly, the respective particles possess quite distinct morphological characteristics and evidenced no propensity to form stable associations in the form of LTP-HDLp complexes. LTP samples were also subjected to low-angle rotary shadowing prior to electron microscopy (**Fig. 2**). These micrographs reveal a similar structure. The apparent indentation in the head region of some LTPs may be the result of flattening by surface tension effects during the vacuum drying procedure.

Trypsin treatment of LTP

The apparent presence of two distinct components in the overall structure led us to examine whether mild proteolysis would permit cleavage and, perhaps, separation of the head and tail sections. If successful, this approach might make it possible to isolate specific fragments that retain lipid binding domains and/or transfer activity. Digestion of LTP with trypsin was followed by analysis of the fragments obtained by SDS-PAGE. Under mild proteolytic conditions it was possible to discriminate the relative

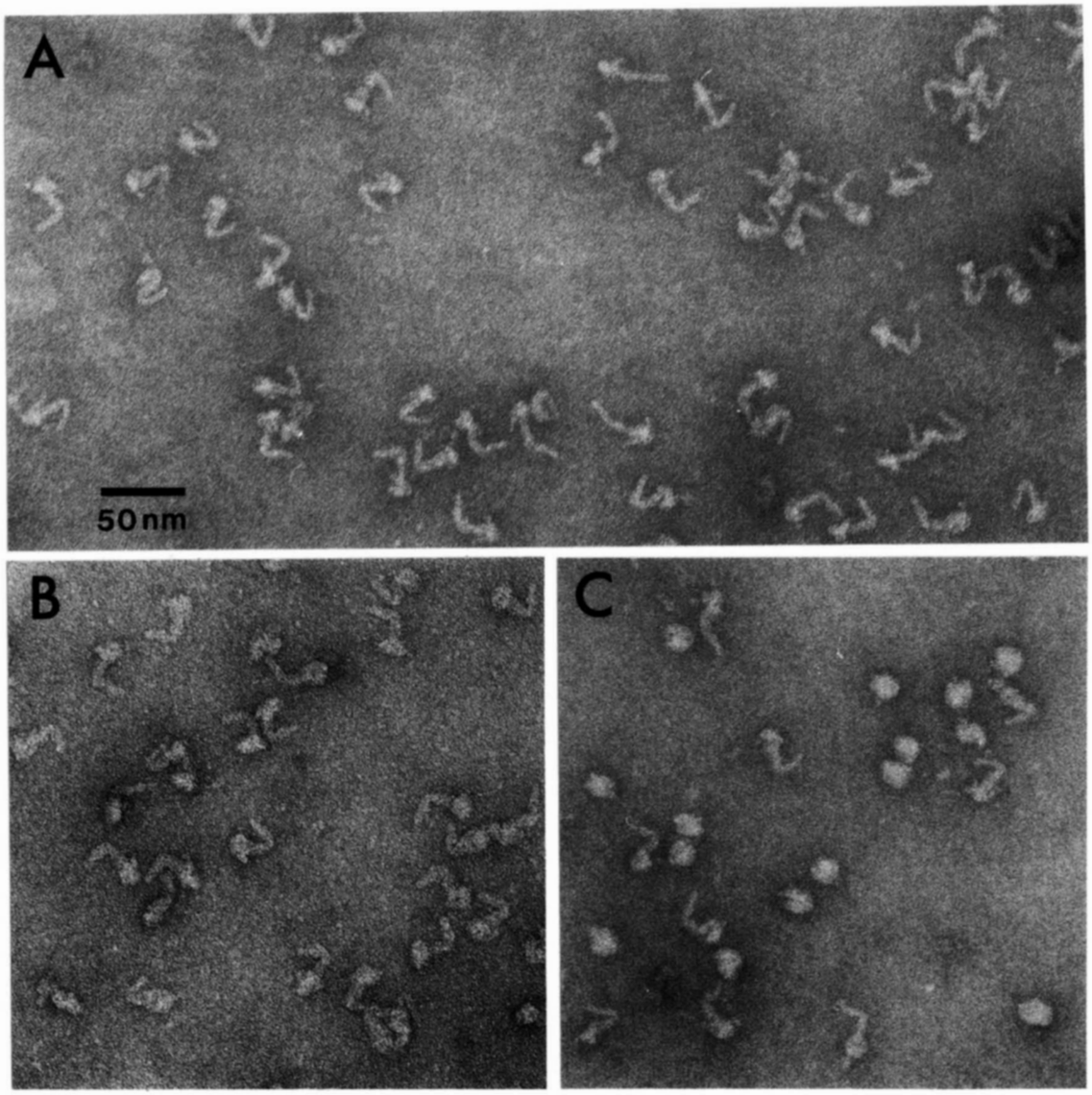

Fig. 1. Electron microscopy of LTP. A: LTP negatively stained with 2% sodium phosphotungstate, B: LTP negatively stained with 1.5% uranyl acetate, and C: micrograph of a sample containing LTP and high density lipophorin (spherical particles, diameter ~17 nm), negatively stained as in A.

susceptibility of the three LTP apoproteins to trypsin digestion. **Fig. 3** shows the pattern obtained for time points ranging from 0 to 120 min at a trypsin–LTP protein ratio of 1:100 (w/w). Control LTP, not incubated with trypsin, possessed the characteristic pattern of three apoproteins (Fig. 3, lane 3). In the trypsin-treated LTP samples, cleavage at the most susceptible site in the native complex resulted in the appearance of a broad band with slightly increased mobility (M_r ~200,000) relative to apoLTP-I, together with a doublet of M_r ~130,000–140,000 (Fig. 3, lane 4). Given the apparent M_r of the proteolytic products and the reported M_r of apoLTP-I (~320,000) it is likely that the observed bands arise from cleavage of apoLTP-I at two distinct but nearby sites. Interestingly, even after 120 min under the conditions of this digestion there was no further proteolysis.

A much different pattern was observed for apoLTP-II. This apoprotein appeared to be resistant to proteolysis at the early times, but by 60 min significant degradation was apparent. The loss of staining intensity of the apoLTP-II band corresponded directly to the appearance of a major proteolytic fragment of M_r 46,000 (Fig. 3, lane 8). Examination of apoLTP-III revealed that the staining intensity of this apoprotein remained constant, suggesting it is resistant to proteolysis under the conditions employed even after 120 min incubation. While it is likely that these results are a reflection of the relative exposure of LTP apoproteins in the native complex, alternative explanations,

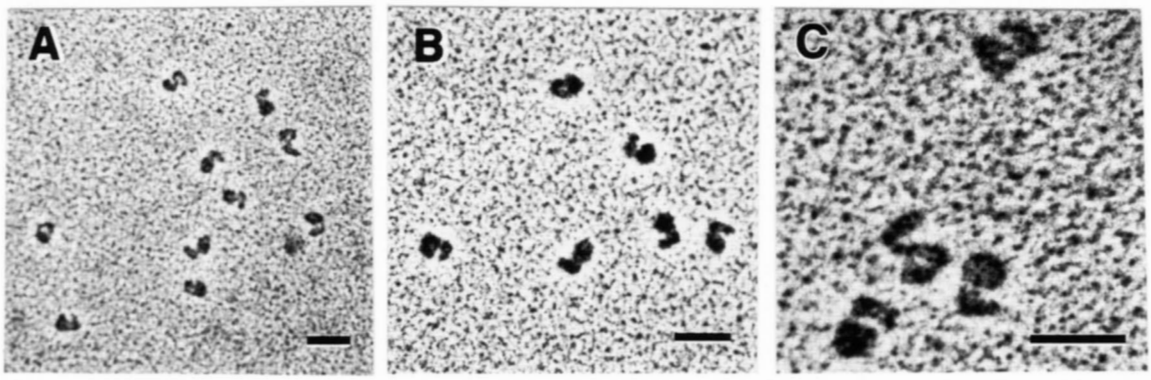

Fig. 2. Low angle rotary shadowing electron microscopy of LTP. Details of sample preparation are provided in the text. Three different magnifications are shown; in each case the bar represents 50 nm.

such as a fortuitous comigration of larger apoprotein fragments to the position of smaller apoproteins, cannot be excluded.

It should be possible, however, to distinguish whether some structural features of the LTP complex impart the apparent resistance to proteolysis by trypsin or whether the specific LTP apoproteins themselves lack trypsin-susceptible sites. This question was addressed by altering the ratio of trypsin to LTP and/or by introduction of SDS into the reaction mixture (**Fig. 4**). As increasing amounts of trypsin were included in the incubation there was a corresponding increase in proteolysis as judged by the electrophoretic patterns obtained. At a 1:1 protein ratio (trypsin–LTP) significant breakdown of all three LTP apoproteins was observed (Fig. 4, lane 5), indicating that each of the three LTP apoproteins contained trypsin-sensitive sites. When a sample of LTP was preincubated at 60°C for 30 min in the presence of 0.1% SDS prior to di-

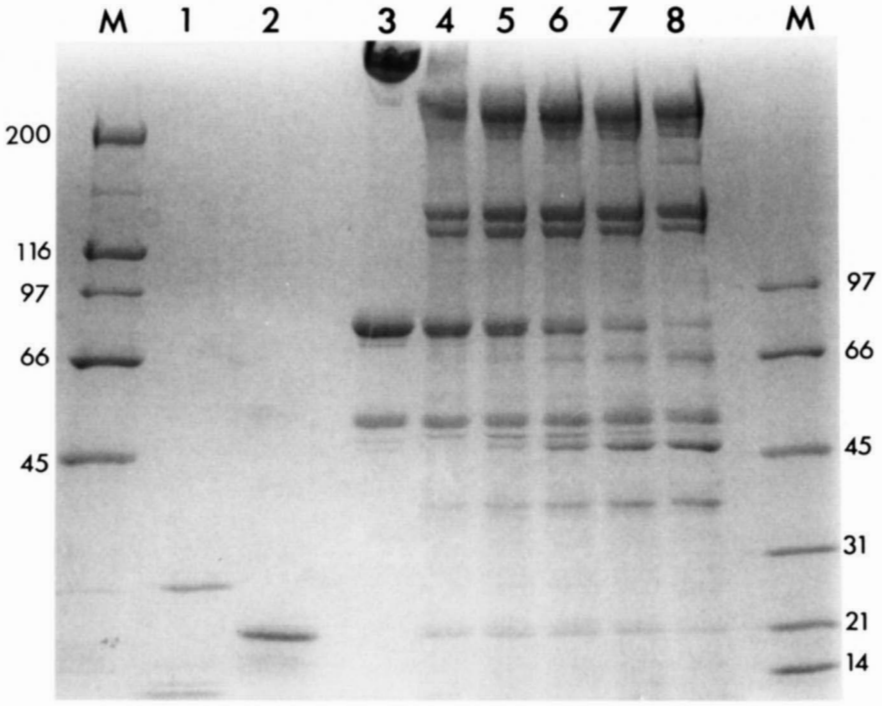

Fig. 3. Effect of trypsin on the electrophoretic pattern of LTP apoproteins. LTP was incubated with trypsin (1:100 w/w; trypsin–LTP protein ratio) for various times at 25°C. After incubation soybean trypsin inhibitor was added and the samples were electrophoresed on a 4–15% acrylamide gradient-SDS slab gel. Lane 1) trypsin, lane 2) soybean trypsin inhibitor, 3) LTP control. Lanes 4–8 represent LTP treated with trypsin for 5 min, 15 min, 30 min, 60 min, and 120 min, respectively. Lanes marked M contained molecular weight markers.

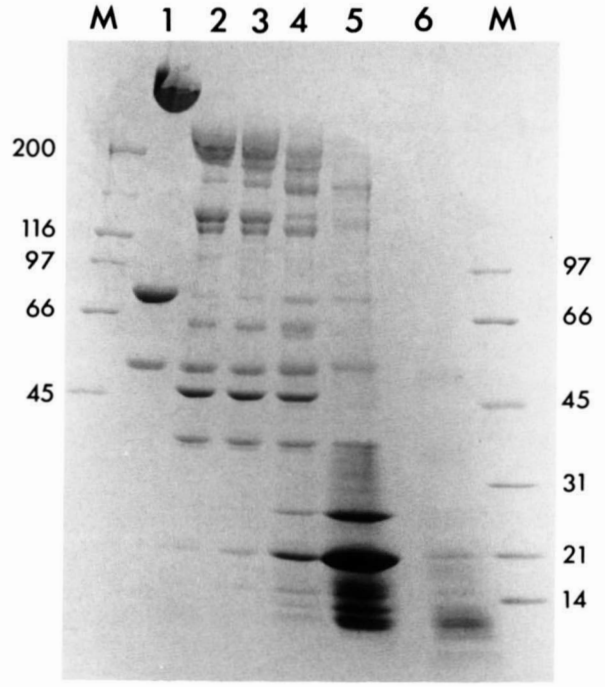

Fig. 4. The effect of trypsin concentration on proteolysis of LTP apoproteins. LTP was incubated with various amounts of trypsin at 25°C for 60 min. After incubation a twofold excess of soybean trypsin inhibitor was added and the samples were electrophoresed on a 4–15% acrylamide gradient-SDS slab gel. Lane M, molecular weight markers; 1) LTP control; 2) 1:100 trypsin–LTP protein; 3) 1:50 trypsin–LTP protein; 4) 1:10 trypsin–LTP protein; 5) 1:1 trypsin–LTP protein; and 6) 1:50 trypsin–LTP protein after a 30-min preincubation in 0.1% SDS (w/v) at 60°C.

gestion with trypsin (trypsin–LTP protein ratio, 1:50) the number of proteolytic fragments (Fig. 4, lane 6) was greater than that observed under the same conditions in the absence of SDS/heat (Fig. 4, lane 3), suggesting that some feature of the apoprotein arrangement in the native complex imparts resistance to proteolysis by trypsin. In subsequent experiments it was observed that the proteolytic pattern obtained when LTP was preincubated with 0.1% SDS for 30 min at 25°C was nearly identical to that obtained at 60°C.

Experiments were also done to determine whether trypsin treatment of intact LTP breaks the particle itself into fragments that can be isolated, or merely introduces nicks in the polypeptide chains without disrupting the tertiary structure. First, trypsinized LTP was subjected to density gradient ultracentrifugation under conditions that cause flotation of the native LTP complex. After centrifugation the tube contents were fractionated and analyzed by SDS-PAGE (**Fig. 5**). The results reveal that trypsinized LTP had flotation properties identical to that of native LTP, even though significant proteolysis had occurred. All bands resulting from proteolysis were found in the same fractions and with similar staining intensities (Fig. 5, lanes 5–8) indicating that the complex remained intact during ultracentrifugation in spite of the action of trypsin. The higher density fractions (Fig. 5, lanes 10–14) showed evidence of only the trypsin and trypsin inhibitor.

We then assayed the catalytic activity of trypsinized LTP, using native LTP as a positive control, to determine

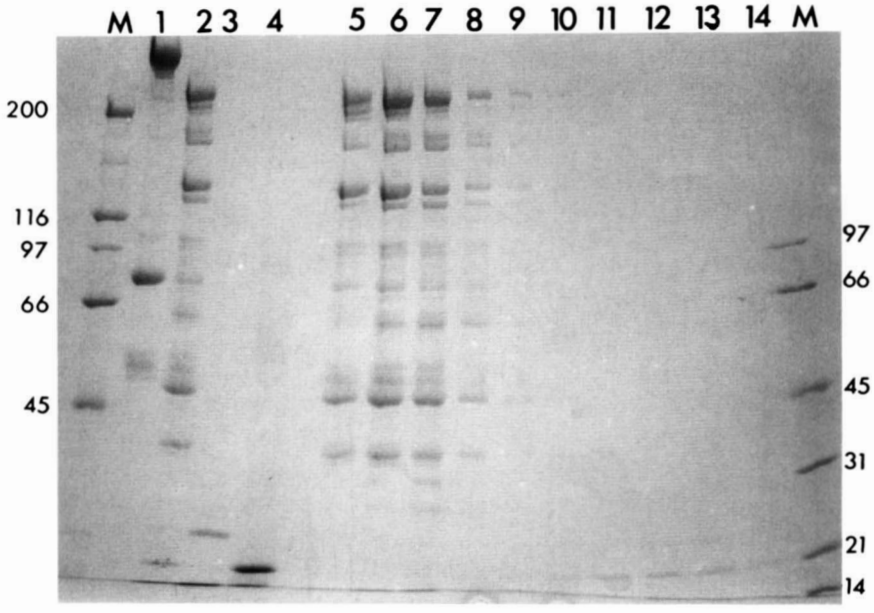

Fig. 5. The effect of trypsin treatment on the flotation properties of LTP. LTP was incubated with trypsin (1:50 trypsin–LTP protein) for 1 h. After incubation soybean trypsin inhibitor was added to stop the reaction. The sample was then subjected to density gradient ultracentrifugation as described in Materials and Methods. After centrifugation the tube was fractionated (0.3 ml) and, after dialysis to remove KBr, an aliquot of each fraction was electrophoresed on a 4–15% acrylamide-SDS slab gel. Lane M) molecular weight markers; lane 1) control LTP; 2) trypsinized LTP; 3) trypsin; 4) soybean trypsin inhibitor. Aliquots of fractions obtained following density gradient ultracentrifugation of trypsinized LTP were electrophoresed in lanes 5–14. The density increases from left to right, and ranges from 1.26 to 1.32 g/ml.

the effect of trypsin on the ability of LTP to facilitate lipid transfer. The assay system used [^{3}H]DAG-labeled HDLp-L as lipid donor and human LDL as acceptor lipoprotein. In the presence of LTP a time- and LTP-dependent net transfer of DAG from HDLp-L into LDL occurred. After separation of donor and acceptor lipoprotein by density gradient ultracentrifugation, determination of the amount of labeled DAG transferred to LDL is a measure of LTP activity. Increasing the amount of trypsin in digestion experiments was accompanied by a corresponding decrease in LTP activity. Greater than half the LTP activity (70 ± 14%, n = 7) remained after a 60-min incubation at a trypsin–LTP protein ratio of 1:50. Interestingly, even at a 1:1 trypsin–LTP protein ratio, where SDS-PAGE revealed significant degradation of all three LTP apoproteins, 25 ± 11% (n = 7) of the transfer activity remained. In contrast, regardless of proteolysis, LTP that was pretreated with 0.1% SDS (25°C) retained only 2 ± 1% (n = 4) of control transfer activity.

Trypsinized LTP was also examined by electron microscopy (**Fig. 6**, panel B). Under conditions (1:50 trypsin–LTP protein ratio) that cause considerable degradation of LTP apoproteins, this micrograph reveals no significant gross morphological alteration when compared to control LTP (Fig. 6, panel A).

Phospholipase-treated LTP

In another approach we treated native LTP with phospholipase A_2 to determine the accessibility of the phospholipid component of LTP (which represents 57% of LTP lipid). Phospholipase A_2 treatment resulted in quantitative conversion of LTP-associated phosphatidylcholine and phosphatidylethanolamine to the corresponding lysophospholipids as determined by thin-layer chromatography. Examination of the treated sample by SDS-PAGE revealed no change in the apoprotein profile, nor was there any loss of transfer activity after phospholipase A_2 treatment (data not shown). Electron microscopy of phospholipase A_2-treated LTP (Fig. 6, panel C) did not reveal any obvious morphological changes. Thus it appears that the lysophospholipids and free fatty acid products of the phospholipase A_2 reaction remain complexed with LTP. This possibility was tested by subjecting phospholipase A_2-treated LTP to density gradient ultracentrifugation. No change in the flotation behavior of phospholipase A_2-treated LTP was observed when compared with control LTP.

The possibility that phospholipase A_2 treatment may alter the trypsin sensitivity of LTP was tested by subjecting phospholipase A_2-treated LTP to trypsin digestion (1:50 trypsin–LTP protein ratio) for 60 min. The phospholipase A_2-treated trypsinized LTP was found to be similar to trypsinized LTP with respect to SDS-PAGE profile, flotation properties, and transfer activity (data not shown). Similarly, electron microscopy revealed no overall

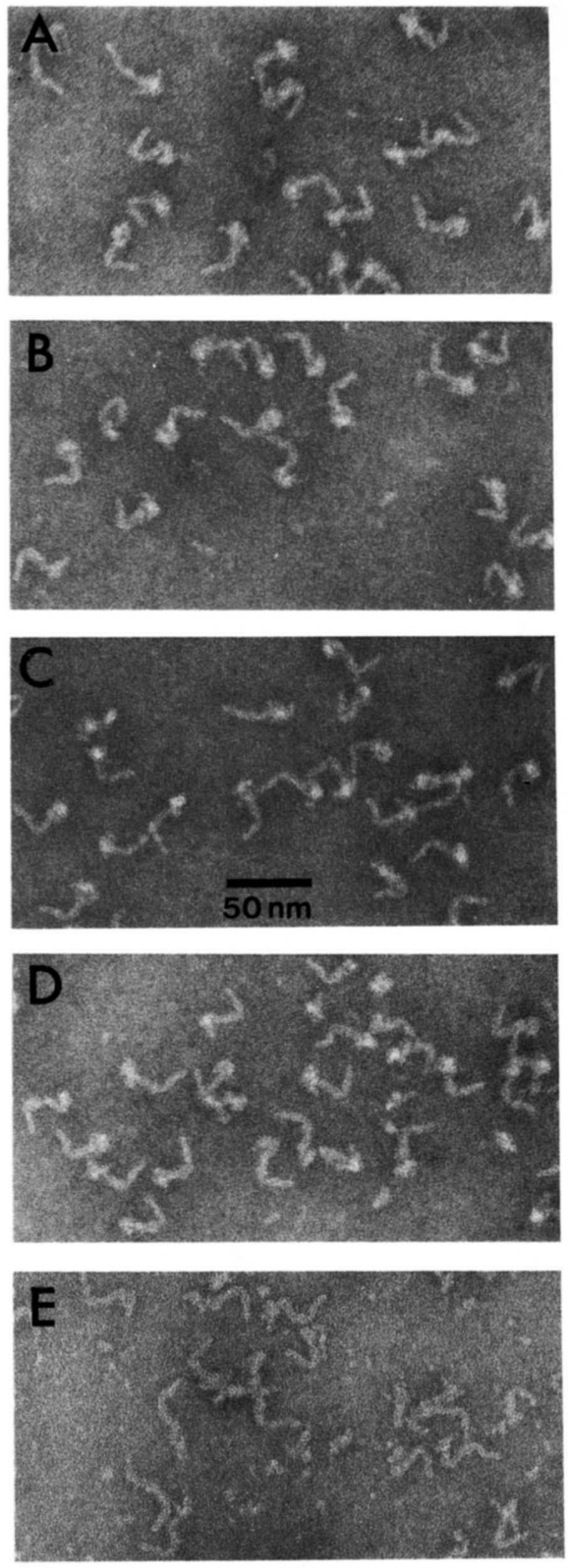

Fig. 6. Electron micrograph of A) control LTP; B) trypsinized LTP (1:50 trypsin–LTP protein); C) phospholipase A_2 (2.5 units/mg LTP)-treated LTP; D) trypsinized, phospholipase A_2-treated LTP; E) LTP in a solution containing 0.1% (w/v) SDS. All samples were negatively stained with 2% sodium phosphotungstate.

structural alterations (Fig. 6, panel D). Finally, although the enzymatic treatments used did not have an observable effect on the structure of LTP as judged by electron microscopy, incubation of LTP with 0.1% SDS at 25°C prior to negative staining did produce significant perturbation (Fig. 6, panel E), with apparent selective destruction of the head region of the particle.

DISCUSSION

M. sexta plasma LTP is unique when compared to other known lipid transfer catalysts (16, 17). It exists as a high molecular weight complex of 14% lipid and three apoprotein components. It is capable of catalyzing exchange and net transfer of several lipid classes among a variety of donor and acceptor species, although it is most efficient in catalyzing transfer of glycerolipids including phospholipid and diacylglycerol. Under appropriate conditions, LTP also promotes net transfer of triacylglycerol (9). By contrast, facilitated transfer of esterified cholesterol occurs much more slowly. (Bae, Y., and R. O. Ryan, unpublished observations). Emerging from studies of LTP lipid class specificity is the concept that the structural properties of the donor and acceptor species are important determinants of the direction, extent, and lipid class specificity of LTP-catalyzed lipid transfer. It has been shown previously that the lipid component of LTP is in equilibrium with the lipid component of donor and acceptor lipoproteins, and that disruption of the complex integrity with detergents abolishes transfer activity (3). In order to understand the mechanism of lipid transfer catalyzed by LTP and to explain its lipid class specificity, more information about the physical structure of the particle is required. We used electron microscopy to visualize the complex and discovered that LTP is an asymmetric particle possessing two distinct structural domains: a roughly spherical head region (~13 nm in diameter) and an elongated (~38 nm) tail (**Fig. 7**). At approximately the midpoint of the tail section, a hinge appears to exist such that the two halves of the tail may assume any angle ranging from 30° to 180° with respect to one another. The significance of this distinctive morphology with respect to substrate binding or lipid transfer is unknown at present, although we hypothesize that the head region of the LTP complex contains most, if not all, of the lipid complement (see below).

Calculations based on the dimensions and density of LTP yield a molecular weight estimate of 1.4×10^6 for the entire complex. In an earlier study (3) after separation of LTP apoproteins by SDS-PAGE, staining with Amido Black 10B, and densitometry, an apoprotein mass ratio of 4.5:1.0:0.34 was obtained for apoLTP-I (M_r ~320,000), apoLTP-II (M_r = 85,000), and apoLTP-III (M_r = 55;000), respectively. This ratio, when taken together with the apoprotein molecular weights, suggests an overall apoprotein stoichiometry of 2:2:1 for apoLTP-I, apoLTP-II, and apoLTP-III, respectively. Given the limitations imposed by this ratio and the molecular weight estimate of 1.4×10^6 from electron microscopy, the combined data are consistent with a holoparticle composition comprised to two apoLTP-I, two apoLTP-II, and one apoLTP-III plus 14% lipid and a molecular weight ~1.0×10^6. Given the asymmetry and unusual shape assumed by LTP, we suggest that the molecular weight estimate determined in the present study can be considered to be in general agreement with the particle composition proposed earlier.

We tested the possibility that mild proteolysis with trypsin might be used to separate structural or functional regions of LTP and provide information about the arrangement of apoproteins in the particle. It was found that mild proteolysis led to the formation of specific fragments that were detectable by SDS-PAGE and which appeared as a function of incubation time and trypsin concentration. Based on the apparent relative susceptibility of the three apoproteins to digestion, it appears that apoLTP-III, the most resistant apoprotein to tryptic digestion, may be shielded from the aqueous environment when present in the intact complex. At higher trypsin–LTP protein ratios or after pretreatment of LTP with 0.1% SDS, all LTP apoproteins were degraded to lower molecular weight fragments. When the flotation properties of trypsinized LTP were examined by density gradient ultracentrifugation it was found that these particles migrated in a manner similar to that of intact LTP. Also, in electron micrographs trypsinized LTP was indistinguishable from untreated control LTP. These results indicate that LTP subjected to partial proteolysis with trypsin retains its tertiary structure as well as its lipid complement, even though its polypeptide apoprotein components have been cleaved at specific sites. It was also found that significant catalytic activity remained after proteolysis. Taken together, these data show that the proteolyzed particle retains its structural integrity. Thus, it is likely that hydrophobic interactions between apoproteins as well as between apoproteins and lipid are important in mainte-

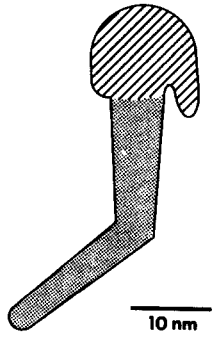

Fig. 7. Representational drawing of *Manduca sexta* LTP. The head portion, which probably contains most, if not all, of the lipid, is rather polymorphic in shape when viewed in the electron microscope; this is an "average" view. The polymorphism is a reflection of structural plasticity. The tail portion, on the other hand, is structurally rigid and consistent in form. The tail does have a hinge at its approximate midpoint, and the two parts can form angles from 30° to 180°. In the drawing, the angle is 130°

nance of the overall structure of the complex. It is not clear, however, whether the active principle of lipid transfer in this system is the entire LTP complex or an apoprotein component that may be separable with retention of activity. Distinguishing between these possibilities must await purification of the individual apoproteins in soluble form. In contrast to trypsinization, SDS treatment of LTP resulted in loss of catalytic activity as well as gross morphological changes as judged by electron microscopy, most notably in the head region of LTP. This observation implies that the lipid complement of LTP resides in this portion of the complex.

Phospholipase A_2 treatment of LTP resulted in conversion of LTP-associated phosphatidylcholine and phosphatidylethanolamine to lysophosphatidylcholine and lysophosphatidylethanolamine, respectively. The quantitative conversion observed indicates that the entire complement of these phospholipids in native LTP is accessible to the enzyme. Phospholipase treatment did not, however, alter the catalytic activity of LTP, affect its flotation behavior, or change its microscopic appearance.

Our experimental results have revealed that insect plasma LTP possesses a unique shape and morphological features that are not measureably altered by limited trypsin digestion or phospholipase treatment. Furthermore, the proteolysis results presented in this report are consistent with a recent report on the effect of protease treatment on human cholesteryl ester transfer protein (18). This M_r 74,000 transfer protein, which also possesses noncovalently associated lipids, retained its native structure and catalytic activity in spite of proteolytic cleavage of its apoprotein backbone. The present work on insect plasma LTP represents the first visualization of a lipid transfer catalyst and raises provocative questions regarding the precise location of LTP apoproteins in the native complex as well as the functional importance of the unusual morphological features evidenced by this catalyst.

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