Lipid transfer particle in the hemolymph of the American cockroach: evidence for its capacity to transfer hydrocarbons between lipophorin particles

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Abstract A lipid transfer particle (LTP) was isolated and purified from the hemolymph of the adult male American cockroach, *Periplaneta americana,* essentially according to the method previously developed for the purification of LTP from locust hemolymph. Fast protein liquid chromatography (FPLC) on a Mono Q column was used as the additional step to obtain pure LTP. The electron micrograph of purified cockroach LTP exhibited an unusual and asymmetric shape essentially similar to that reported for *Manduca sexta* LTP (Ryan et al. 1990. *J. Lipid Res.* **31:** 871–879). The cockroach LTP was also found to be basically similar to that of *M. sexta* and locust in terms of subunit structure and lipid composition, although there were significant differences particularly in the contents of hydrocarbons and diacylglycerol. A simple method for assaying LTP activity was developed, based on the finding that cockroach LTP can catalyze the transfer of labeled hydrocarbons (or diacylglycerol) from labeled high density lipophorin (HDLp) bound with a transfer membrane (Immoblion) to unlabeled HDLp dissolved in saline. This finding reveals that cockroach LTP has the capacity to transfer and/or exchange lipids between lipophorin particles with the same density. It was also demonstrated that cockroach LTP has the capacity to catalyze the transfer and/or exchange of hydrocarbons, in addition to diacylglycerol, between cockroach HDLp and locust low density lipophorin (LDLp).—**Takeuchi, N., and H. Chino.** Lipid transfer particle in the hemolymph of the American cockroach: evidence for its capacity to transfer hydrocarbons between lipophorin particles. *J. Lipid Res.* 1993. **34:** 543–551.

Supplementary key words diacylglycerol • high density lipophorin • low density lipophorin • locust • *M. sexta*

Lipophorin (lipid-bearing protein) probably exists in the hemolymph of all species of insects and serves to shuttle diacylglycerol, cholesterol, and hydrocarbons between tissues (1, 2). An insect lipid transfer particle (LTP) was first isolated by Ryan et al. (3, 4) from the larval hemolymph of the tobacco hornworm, *Manduca sexta.* LTP has been shown to have a capacity to catalyze the net transfer and/or exchange of diacylglycerol between lipophorin particles having different densities, i.e., low density lipophorin (LDLp) and high density lipophorin (HDLp), resulting in a new intermediate density lipophorin (3, 4). Evidence that LTP also promotes the loading of diacylglycerol by lipophorin from the fat body has been provided by Van Heusden and Law (5). Another function of LTP has been demonstrated recently; LTP has the capacity to facilitate the transfer of diacylglycerol between insect lipophorin and human plasma lipoprotein (6). The subunit structure and lipid composition of *M. sexta* LTP has been characterized (7). The LTP is composed of three apoproteins with the following molecular weights: apoLTP-I, 320,000; apoLTP-II, 85,000; and apoLTP-III, 55,000. LTP is a lipoprotein with 14% lipids comprised of phospholipid, diacylglycerol, free fatty acid, and triacylglycerol (7). Furthermore, Ryan et al. (7) have provided evidence that the lipid component (e.g., diacylglycerol) of LTP is involved in the function of LTP.

All the above reports strongly suggest that LTP plays an important role in facilitating the function of lipophorin. As lipophorin exists in the hemolymph of almost all insect species, it is also conceivable that LTP resides not only in the hemolymph of *M. sexta* but also in the hemolymph of other insects. In fact, we have recently demonstrated the existence of LTP in the hemolymph of the locust, *Locusta migratoria* (8) and we have shown that locust LTP is essentially similar to that of *M. sexta* LTP in terms of physicochemical properties and function (8). We have also demonstrated that locust LTP contains a significant amount of hydrocarbons in addition to phospholipid, diacylglycerol, cholesterol, and free fatty acid (8). One of the important functions of lipophorin is the transport of hydrocarbons from the oenocytes, which are the sites of hydrocarbon synthesis, to the cuticle where hydrocarbons are deposited as cuticular wax (9). It is, therefore, very

Abbreviations: LTP, lipid transfer particle; HDLp, high density lipophorin; LDLp, low density lipophorin; AKH, insect adipokinetic hormone; PMSF, phenylmethylsulfonyl fluoride; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; FPLC, fast protein liquid chromatography.

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probable that LTP has the capacity to catalyze the transfer not only of diacylglycerol but also of hydrocarbons between lipophorin particles. Recently, Amareshwar Singh and Ryan (10) have reported that *M. sexta* LTP has the ability to catalyze the transfer of hydrocarbons from *M. sexta* lipophorin (HDLp) to human low density lipoprotein. However, there still remains the important question as to whether LTP can mediate the transfer of hydrocarbons between insect lipophorin particles.

In this study, we will report the isolation of LTP from the hemolymph of the adult American cockroach, *Periplaneta americana,* and will provide several lines of evidence to demonstrate the capacity of cockroach LTP to transfer and/or exchange hydrocarbons, in addition to diacylglycerol, between lipophorin particles. We will also describe a new and simple method for assaying LTP activity.

MATERIALS AND METHODS

Animal and hemolymph collection

Adult male American cockroaches, *Periplaneta americana,* and adult male locusts (3–6 weeks after final molt), *Locusta migratoria,* were taken from colonies maintained in this laboratory. Locusts were fed on pampas and orchard grass. Cockroaches were reared on dog biscuits and boiled carrots to give a yellow coloration to lipophorin (11). Hemolymph was collected from cockroaches or locusts by flushing the hemocoele with buffered saline (0.15 M NaCl, 5 mM EDTA, PIPES buffer, pH 6.8). The pooled hemolymph was centrifuged at 2,000 *g* for 5 min to remove the hemocytes.

Chemical

Insect adipokinetic hormone (AKH) was obtained from Peninsula Laboratories (San Carlos, CA). The following standard marker proteins were obtained from Bio-Rad Laboratories (Richmond, CA); myosin (200,000), β-galactosidase (116,250), phosphorylase b (97,400), bovine serum albumin (66,200), ovalbumin (42,700). DEAE-Sepharose CL-6B and transfer membrane (Immoblion) were purchased from Pharmacia and Millipore, respectively. Sodium [1-^{14}C]acetate (2.9 mCi/mmol) and [1-^{14}C]palmitic acid (50 mCi/mmol) were obtained from New England Nuclear. Double glass-redistilled water was used throughout.

Preparation of lipophorin

HDLp was prepared from the hemolymph of male cockroaches or male locusts by KBr-density gradient ultracentrifugation essentially according to Shapiro, Keim, and Law (12) as described by Chino, Downer, and Takahashi (13). After centrifugation, the yellow lipophorin fraction was collected by a Pasteur pipette. LDLp was prepared from the hemolymph of male locusts 60 to 90 min after AKH injection by the same density gradient ultracentrifugation method. In some experiments, a specific precipitation method of Chino and Kitazawa (14) was used to prepare HDLp. All HDLp and LDLp preparations were dialyzed several times against insect Ringer (120 mM NaCl, 15 mM KCl, 4 mM $CaCl_2$, 2 mM $MgCl_2$, 5 mM PIPES buffer, pH 7.0) before use in incubation experiments.

Labeling of hydrocarbons or diacylglycerol associated with cockroach lipophorin

In order to label the hydrocarbons of cockroach lipophorin, sodium [1-^{14}C]acetate ($1 \cdot 10^6$ dpm/insect) was injected into male cockroaches 4 h before hemolymph collection. Hydrocarbons are the predominantly labeled lipid of lipophorin isolated from injected cockroaches (75–80%) while the label found in diacylglycerol is less than 10% (9). To label diacylglycerol of cockroach lipophorin, male cockroaches were fed a solution containing [1-^{14}C]palmitic acid ($1 \cdot 10^6$ dpm/insect), as described in an earlier paper (15), 120 min before hemolymph collection. The label in lipophorin isolated from the hemolymph of the [1-^{14}C]palmitic acid-fed cockroaches is almost exclusively associated with the diacylglycerol fraction (more than 94%) while only a trace (less than 0.3%) of the label is found associated with the hydrocarbons (16).

Assay method for lipid transfer particle

The assay method for locust LTP described in the previous paper (8) is based on the measurement of radioactivity of diacylglycerol transferred from labeled HDLp (or LDLp) as donor to unlabeled LDLp (or HDLp) as acceptor during incubation. This method is rather complicated and involves several steps including the preparation of LDLp and KBr-density gradient ultracentrifugation. However, because hemolymph of the American cockroach lacks apolipophorin-III (11), an essential component in the transformation of HDLp to LDLp (17), it is not possible to prepare cockroach LDLp by the injection of AKH. In addition, as will be described in Results, it was found that cockroach LTP has the capacity to transfer not only diacylglycerol but also hydrocarbons between lipophorin particles. Thus, we have exploited this novel property to develop a simpler method for assaying the activity of LTP.

Step 1. Preparation of the labeled HDLp bound with transfer membranes

Two pieces of transfer membrane (Immoblion, pore size: 0.45 nm, diameter: 2 cm) were incubated in a small beaker with hydrocarbon-labeled HDLp (about 1 mg protein) in 1 ml insect Ringer at 30°C for 2 h with constant shaking. During incubation, a fine glass rod was placed between the two membranes to prevent the membranes

from sticking together. After 2 h of incubation, about 150 μg protein of HDLp was found associated with each membrane. The two membranes were then incubated at 4°C for 2 h with constant shaking in a 1 ml solution of 0.05% Tween 20, 150 mM NaCl, and 20 mM Tris-HCl buffer, pH 7.4, to block the membranes. The membranes were then washed three times with excess insect Ringer to remove unbound HDLp from the membrane. The consistency of the labeled HDLp-bound membrane was tested by determining the radioactivity associated with the membrane. The result indicated that all membranes contain practically the same radioactivity as long as the membranes are prepared by incubation in the same source of labeled HDLp.

Step 2. Incubation of the labeled HDLp-bound membrane with unlabeled HDLp

Two membranes prepared as above were incubated in a small beaker containing unlabeled HDLp (1 mg protein) in 1 ml insect Ringer with the LTP fraction (200 μl) at 30°C for 2 h with constant shaking. After incubation, 1 ml of the incubation medium was subjected to radioassay. Incubation without the LTP fraction was run as a control at the same time. Four membranes were needed for each set of experiments. At times, incubation of the membrane with saline alone was run to check spontaneous release of labeled HDLp from membrane into saline. A typical result of the time course of the transfer of labeled hydrocarbons from HDLp bound with the membrane to unlabeled HDLp under the above conditions is illustrated in **Fig. 1** and demonstrates that the transfer takes place continuously up to 2 h in the presence of LTP while practically no transfer occurs throughout the incubation period without the addition of LTP. Furthermore, fractionation using Florisil column chromatography (18) of lipids extracted from the incubation medium after a 2-h incubation and the subsequent radioassay indicated that radioactivity recovered in the incubation medium associates exclusively with the hydrocarbon fraction. All the results reveal that the assay method developed in the present study is useful, and is the method used in this study to monitor cockroach LTP throughout the purification procedures.

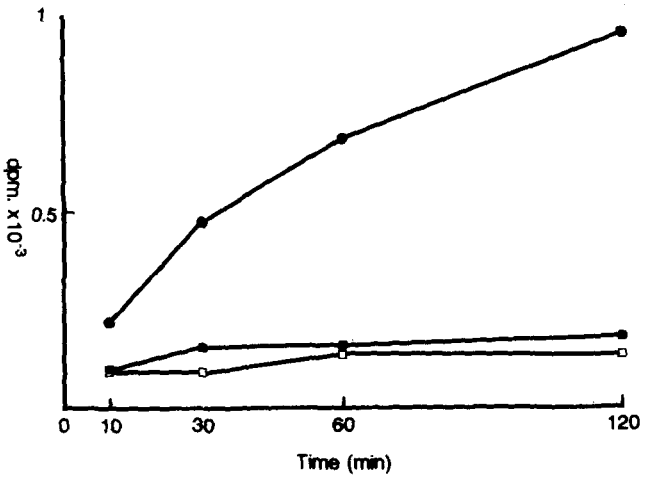

Fig. 1. Time course of radioactive hydrocarbon transfer from labeled HDLp bound with transfer membrane as donor to unlabeled HDLp as acceptor in incubation medium. In each incubation, two transfer membranes containing about 350 μg protein and about 5,000 dpm hydrocarbon were incubated with the following incubation media: □, insect Ringer only; ■, unlabeled HDLp (500 μg protein); ●, the mixture (500 μg protein) of unlabeled HDLp and LTP (the fraction precipitated under low ionic concentration, which comprises both HDLp as the major protein and LTP as a minor protein; see purification procedures of LTP in Methods). The incubation medium was brought to 1.2 ml final volume by adding Ringer solution. An aliquot (1 ml) was taken at intervals and assayed for radioactivity. Ordinate indicates radioactivity found in 1 ml incubation medium. Four incubations were run to obtain data for each curve. Accordingly, twelve incubations in total were run for one set of experiments.

Purification of lipid transfer particle

The purification procedures used in this experiment were essentially similar to the method developed for the purification of locust LTP in a previous study (8). The process consisted of three steps; specific precipitation under low ionic concentration, DEAE-Sepharose column chromatography, and KBr density gradient ultracentrifugation.

In this study, hemolymph (about 50 ml containing 550–600 mg protein) collected and pooled from 200 male cockroaches was used as the starting material. Unless otherwise stated, all procedures were done at 2°C. The hemolymph was first dialyzed against large volumes of distilled water until the hemolymph solution became slightly turbid. After removing the turbidity by centrifugation at 10,000 *g* for 5 min, 8 volumes of ice-cold distilled water was rapidly added to the supernatant in order to precipitate lipophorin and small amounts of other proteins including LTP. The precipitate dissolved in a small volume of saline (0.1 M NaCl, 1 mM EDTA, 1 mM PMSF, 10 mM PIPES buffer) was subjected to DEAE-Sepharose CL-6B column chromatography. The column was first run with 100 ml of the same saline to elute lipophorin, and subsequently run with a linear concentration gradient of 0.1–0.4 M NaCl (in saline). The LTP activity was found in the fractions eluted with 0.15–0.25 M NaCl, as in the case of locust LTP (8). To the combined active fractions (about 20 ml in total) solid KBr was added to give a final density of 1.31 g/ml. The solution was placed into centrifuge tubes and overlayered with a equal volume of KBr–saline (1.10 g/ml). The tubes were then centrifuged at 50,000 rpm for 16 h at 4°C in an Hitachi ultracentrifuge (model 70P-72). After centrifugation, the sample was fractionated from the top and an aliquot of each fraction was assayed for LTP activity. Unlike the locust LTP which appeared at d 1.22 g/ml (8), the cockroach LTP fraction appeared at d 1.20–1.22 g/ml as a rather broad band in the KBr density gradient ultracentrifuga-

tion step, suggesting that the fraction is still contaminated with other proteins. In fact, the SDS-PAGE of the cockroach LTP at this step displayed several bands on the gel. Therefore, FPLC on a Mono Q column (Pharmacia) was undertaken as an additional step to eliminate the remaining contaminating proteins. The LTP fraction obtained after the KBr density gradient ultracentrifugation step was first dialyzed against a large volume of saline (0.1 M NaCl, 50 μM PMSF, 10 mM PIPES buffer, pH 6.2), and applied to the Mono Q column that had been equilibrated with the same saline. After eluting with the same saline for 40 min (flow rate 1 ml/min), the column was then eluted with a linear concentration gradient of 0.1–0.4 M NaCl (in saline). The elution profile is shown in **Fig. 2** and demonstrates that the LTP fraction appears as a single peak at 0.28–0.33 M NaCl. No significant LTP activity was detected in other peak fractions. The final purification step yielded 60 μg protein of cockroach LTP. This value was much lower compared to the yield of the locust LTP (160 μg LTP from 400 mg hemolymph protein) at the KBr density gradient ultracentrifugation step, which gave pure LTP in the case of the locust (8). Whether the low yield of cockroach LTP is due to the original low concentration of LTP in the hemolymph or to the purification procedures is unknown, because the recovery of LTP was not determined in this study.

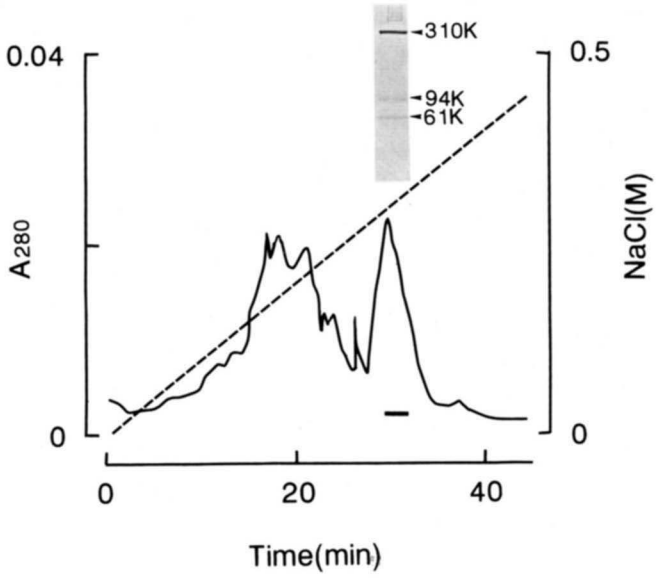

Fig. 2. A linear concentration gradient elution profile (FPLC on Mono Q column) of the LTP fraction obtained by KBr density gradient ultracentrifugation. The column was run at a flow rate of 1 ml/min. After eluting with 40 ml of saline (0.1 M NaCl, 50 μM PMSF, 10 mM PIPES buffer, pH 6.2), the column was eluted with a linear concentration gradient of 0.1–0.4 M NaCl in saline. The horizontal bar represents the fraction with LTP activity. No significant LTP activity was detected in other peak fractions. The insert shows the SDS-PAGE of the fraction with LTP activity.

Lipid determination

Lipids were extracted from purified LTP with chloroform–methanol 2:1 (v/v) and analyzed by an Iatroscanner (model Th-10) essentially according to the method of Ackman (19) as described by Chino, Kiyomoto, and Takahashi (17).

Gas–liquid chromatography of hydrocarbons

The lipids from cockroach HDLp or locust LDLp, extracted with chloroform–methanol as above, were first subjected to Florisil column chromatography (18) to separate the hydrocarbon fraction. The hydrocarbon fraction was then subjected to gas–liquid chromatography using a Shimadzu model GM-4M. A glass column (3 m × 3 mm) containing 1.5% OV-17 on Chromosorb W was used with N_2 as carrier gas, and programmed from 210 to 230°C at 1°C per min.

Gel electrophoresis

SDS-polyacrylamide gel electrophoresis of purified LTP was performed according to the method of Laemmli (20). The gels were stained with Coomassie blue.

Electron microscopy

The purified cockroach LTP was applied to a supporting film, stained with 1% uranyl acetate, and observed in a JEOL (model JEM-1200EX) electron microscope. The supporting films coated with carbon were made hydrophilic by ion bombardment before use.

Determination of protein amount and radioactivity

Protein was determined by the method of Lowry et al. (21). Radioactivity was counted in a liquid scintillation counter (LKB, model 1219) after dissolving the samples in Aquasol-2.

RESULTS

Partial characterization of cockroach LTP

A negatively stained electron micrograph of the purified cockroach LTP is illustrated in **Fig. 3**, and demonstrates an unusual asymmetric shape. The images are essentially similar to those reported for *M. sexta* LTP by Ryan, Howe, and Scraba (22), and exhibit two major structural features: a roughly spherical head and an elongated tail with a hinge.

The subunit structure of LTP was examined by SDS-PAGE. As seen in Fig. 2 (insert), cockroach LTP is composed of three apoprotein components, as reported for *M. sexta* LTP (7) and locust LTP (8); apoLTP-I (mol wt, 310,000), apoLTP-II (mol wt, 94,000), and apoLTP-III (mol wt, 61,000). The estimated molecular weights of the three apoproteins are almost equivalent to the respective

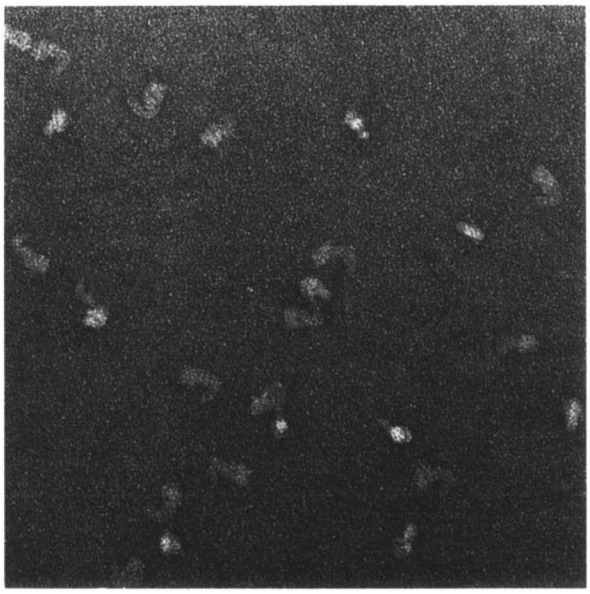

Fig. 3. Electron micrograph of cockroach LTP purified by FPLC on a Mono Q column. The sample (10 μg protein/ml) was applied to a supporting film and stained with 1% uranyl acetate. Magnification: × 200,000.

values reported for *M. sexta* LTP and locust LTP, although a significant difference exists particularly in apoLTP-II: 85,000 for *M. sexta* apoLTP-II, 89,000 for locust apoLTP-II, and 94,000 for *P. americana*.

The lipid compositions of cockroach LTP are given in **Table 1** together with the data of locust LTP, cockroach lipophorin, and locust lipophorin as references. The cockroach LTP contains more lipid than locust LTP. The biggest difference between the two LTPs is the content of hydrocarbon and diacylglycerol; the cockroach LTP contains a large amount of hydrocarbon with a very small content of diacylglycerol; on the other hand, diacylglycerol is the major neutral lipid in the locust LTP. This appears to reflect the difference in the lipid composition between cockroach lipophorin and locust lipophorin, although the extremely low diacylglycerol content of the cockroach LTP is remarkable.

Function of cockroach LTP

As demonstrated in Methods, it became clear that cockroach LTP has the capacity to transfer hydrocarbons from membrane-bound HDLp to HDLp in solution. We then tested whether cockroach LTP can mediate the transfer of diacylglycerol between HDLp bound to the membrane and HDLp in solution, and also examined the relative transfer rates of hydrocarbon and diacylglycerol by cockroach LTP. The typical results given in **Fig. 4** demonstrate that the transfers of both hydrocarbon and diacylglycerol mediated by LTP proceed steadily for at least 60 min of incubation, and that hydrocarbons are transferred more rapidly than diacylglycerol. The difference observed between hydrocarbon and diacylglycerol becomes more pronounced when the transfer rates are expressed as the absolute amount of the two lipids transferred during a certain incubation period. As the two membranes used for each incubation contain approximately 300 μg protein, 2.8 μmol of hydrocarbon and 0.96 μmol diacylglycerol are calculated to have been originally contained in the two membranes, based on the previous data of the lipid compositions of cockroach HDLp (16). Thus, under the conditions described in Fig. 4, 330 nmol of hydrocarbons is calculated to be transferred during a 60-min incubation, while only 75 nmol of diacylglycerol is transferred during the same incubation period.

We have reported in earlier papers (14, 23) that insect lipophorin does not exhibit species specificity in terms of its function; lipophorins from locust and cockroach can accept diacylglycerol or hydrocarbons from the fat body or the oenocytes of the other insect species. Furthermore, the recent studies on *M. sexta* LTP by Ryan and his co-

TABLE 1. A typical analysis of lipid composition of cockroach LTP. The data of locust LTP (8), cockroach, and locust lipophorins (13) are also shown for comparison.

	Weight %			
Components	Cockroach LTP	Locust LTP	Cockroach Lipophorin	Locust Lipophorin
Protein	79	86	50	59
Total lipids	21	14	50	41
1 Hydrocarbon	40	17	28	21
2 Triacylglycerol	ND	ND	2	2
3 Diacylglycerol	2	44	15	33
4 Cholesterol	trace	8	5	8
5 Phospholipids	57	18	43	36
6 Free fatty acid	ND	13	ND	ND
Sum of 1–6	99	100	93	100

Lipid fractions (1–6) are expressed as percentages of the total lipids; ND, not determined.

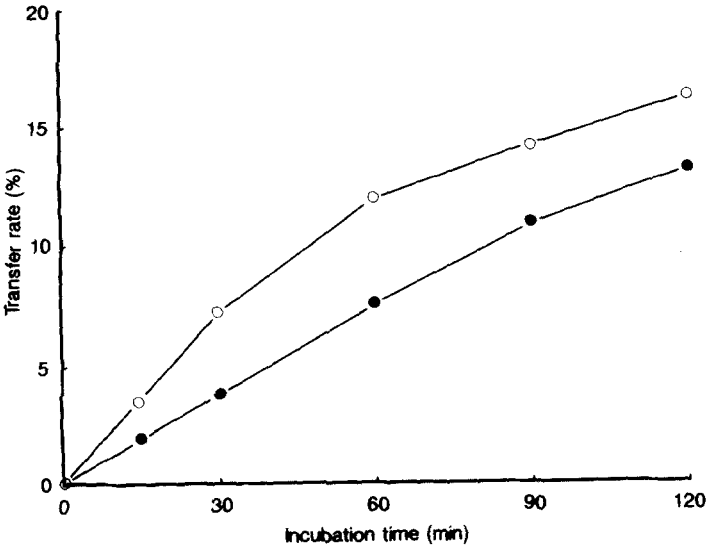

Fig. 4. The relative transfer rates of hydrocarbon and diacylglycerol by cockroach LTP. In each incubation, two transfer membranes binding hydrocarbon-labeled HDLp (307 ± 4.7 μg protein and 6,980 ± 102 dpm hydrocarbon, the mean ± SD of three determinations) or binding diacylglycerol-labeled HDLp (296 ± 12.5 μg protein and 25,150 ± 330 dpm diacylglycerol) were incubated with unlabeled HDLp (1 mg protein) in 1 ml insect Ringer. The reaction was started by adding 200 μl (20 μg protein) of LTP partially purified at the step of DEAE-Sepharose column chromatography. An aliquot (1 ml) of incubation media was taken at intervals and assayed for radioactivity. The transfer rates are expressed as percentage of the radioactivity found in incubation medium against the original radioactivity bound to the two transfer membranes. Each value was corrected for the radioactivity found in the control incubation medium without LTP; ○, hydrocarbon; ●, diacylglycerol.

workers (6) reveal a broad spectrum concerning donor and acceptor lipoproteins; e.g., *M. sexta* LTP can mediate diacylglycerol transfer between lipophorin and human low density lipoprotein (24). As a preliminary experiment to test the versatility of cockroach LTP, we incubated diacylglycerol-labeled cockroach HDLp with unlabeled locust LDLp in the presence of cockroach LTP. The results showed that labeled diacylglycerol was certainly being transferred from cockroach HDLp to locust LDLp during incubation (data not shown). We then tested whether cockroach LTP has the capacity to transfer hydrocarbons between cockroach HDLp and locust LDLp. The hydrocarbon-labeled cockroach HDLp was incubated with the unlabeled locust LDLp in the presence of cockroach LTP. After a 3-h incubation, the incubation medium was subjected to KBr-density gradient ultracentrifugation. After centrifugation for 4 h, the tubes were fractionated from the top, and each fraction was monitored at 280 nm and radioassayed. The results are given in **Fig. 5.** It is clearly demonstrated that labeled hydrocarbons were transferred from labeled cockroach HDLp to unlabeled locust LDLp when the cockroach LTP was present. Approximately 30% of labeled hydrocarbons originally present in cockroach HDLp were transferred to locust LDLp during a 3-h incubation. Contrary to this, when the incubation was run in the absence of cockroach LTP, no transfer was observed, indicating that spontaneous transfer of hydrocarbons did not occur, at least during the 3-h incubation.

We have reported in earlier papers (14, 16) that the hydrocarbon compositions of cockroach lipophorin differ largely from those of locust lipophorin; the former comprises only three hydrocarbons, n-pentacosane, 3-methylpentacosane, and 6,9-heptacosadiene, while the latter is composed of a number of saturated hydrocarbons ranging in chain lengths from C_{25} to C_{38}. In order to provide further evidence for the transfer of hydrocarbons from cockroach HDLp to locust LDLp by the action of cockroach LTP, we analyzed the hydrocarbon composition of locust LDLp, which had been incubated with cockroach HDLp in the presence or absence of cockroach LTP. After isolation of locust LDLp by density gradient ultracentrifugation from the incubation medium, the lipids were extracted from locust LDLp with chloroform–methanol 2:1 (v/v) and then applied to a Florisil column to separate the hydrocarbon fraction, which was then analyzed by gas–liquid chromatography. The chromatograms illustrated in **Fig. 6** clearly demonstrate that three hydrocarbons, n-pentacosane, 3-methylpentacosane, and 6,9-heptacosadiene, which are intrinsically absent from locust lipophorin, appear in locust LDLp after incubation with cockroach HDLp in the presence of LTP, revealing that the three hydrocarbons are certainly transferred from cockroach HDLp to locust LDLp under the action of cockroach LTP. The reverse transfer of hydrocarbons, i.e., from locust LDLp to cockroach HDLp, was also tested by the analysis of hydrocarbon compositions of the latter after incubation with the former in the presence of cockroach LTP. The results (**Fig. 7**) clearly demonstrate that a number of saturated hydrocarbons became associated with cockroach HDLp, strongly suggesting that the observed transfer of hydrocarbons, mediated by cockroach LTP, between locust LDLp and cockroach HDLp is an exchange reaction.

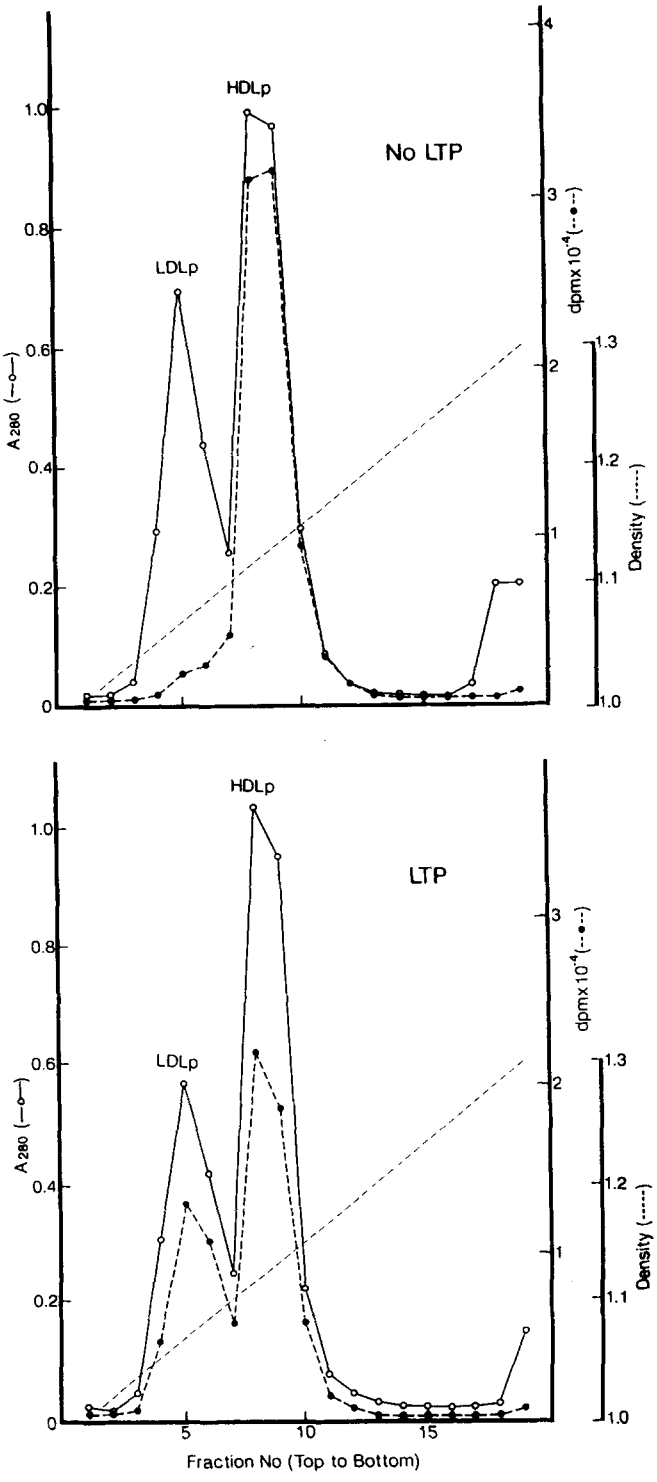

Fig. 5. Transfer of labeled hydrocarbons from cockroach HDLp to locust LDLp. Labeled cockroach HDLp (0.8 ml) containing 2.4 mg protein and about 90,000 dpm hydrocarbons was incubated with 1 ml unlabeled locust LDLp (1.56 mg protein) in the presence of 200 μl of purified LTP (1.5 μg protein) at 30°C for 3 h. After incubation, the incubation medium was brought to 5 ml by adding saline and subjected to KBr density gradient ultracentrifugation. After centrifugation for 4 h, the solutions in tubes were fractionated in 0.5 ml fractions from the top. The lipophorin fractions were monitored at 280 nm, and the radioactivity in each fraction was determined.

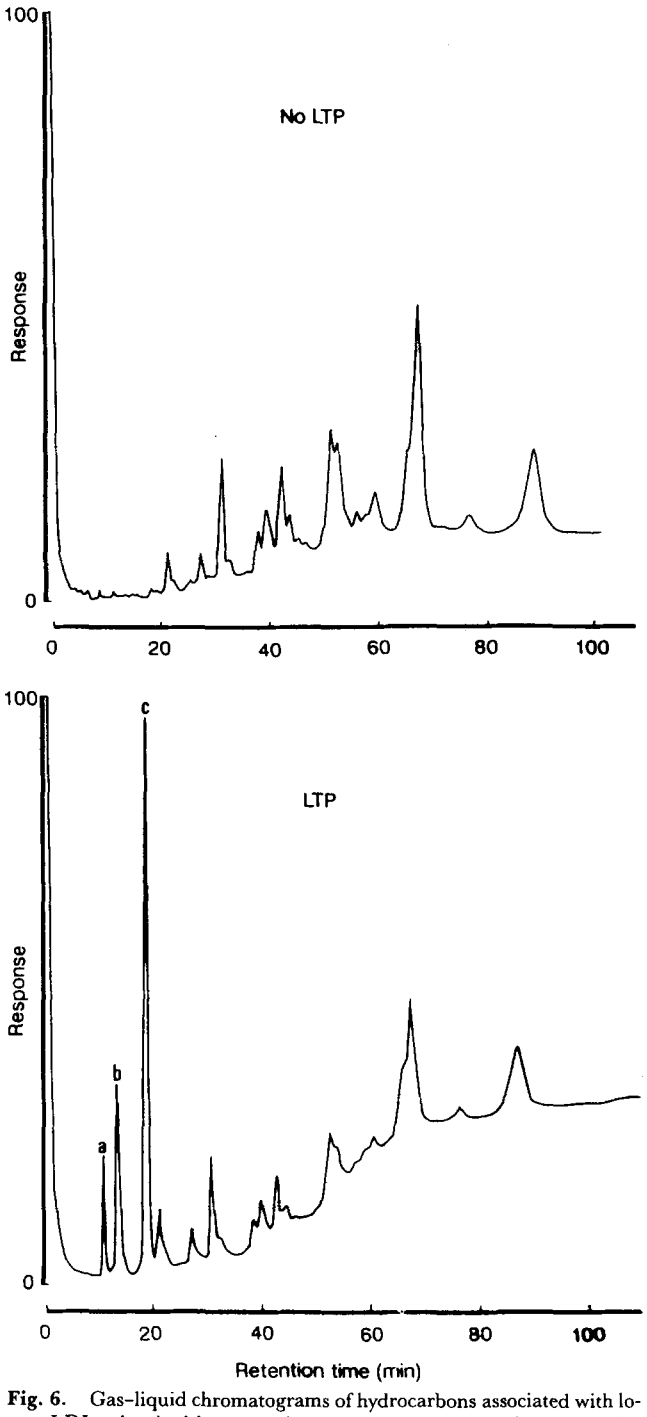

Fig. 6. Gas-liquid chromatograms of hydrocarbons associated with locust LDLp that had been previously incubated with cockroach HDLp in the absence or presence of cockroach LTP; a: n-pentacosane; b: 3-methylpentacosane; c: 6,9-heptacosadiene.

DISCUSSION

All the experimental data presented in this study provide definite evidence that cockroach LTP can mediate the transfer and/or exchange of hydrocarbons, in addition to diacylglycerol, between lipophorin particles. Further-

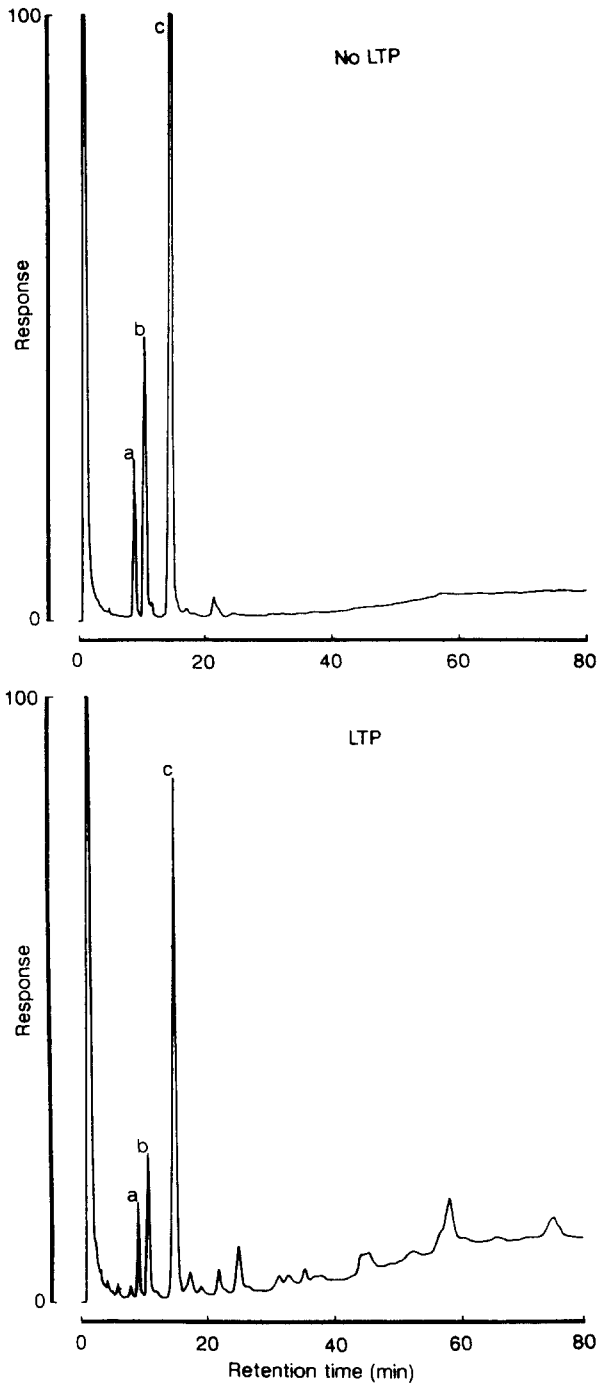

Fig. 7. Gas-liquid chromatograms of hydrocarbons associated with cockroach HDLp previously incubated with locust LDLp in the absence or presence of cockroach LTP. Other explanations as in Fig. 6.

more, there is no functional species specificity, suggesting that cockroach LTP has a broad spectrum concerning donor and acceptor lipoproteins as reported for *M. sexta* LTP (24). The data also support that cockroach LTP is essentially similar to LTP from *M. sexta* and locusts, in terms of physicochemical properties and function.

In several studies of *M. sexta* LTP (4, 5, 7) and locust LTP (8), the function of LTP has been focused on the transfer and/or exchange of lipid, such as diacylglycerol, between lipophorin particles with different densities, i.e., LDLp and HDLp. However, the basic question as to whether LTP can catalyze the transfer of such lipids between lipophorin particles with the same density has been left unanswered. The method developed in the present study for assaying the LTP activity is based on LTP-mediated hydrocarbon transfer between HDLp in solution and HDLp bound with a transfer membrane. Although there is no direct evidence that the membrane-bound HDLp still retains its native properties, the fact that the above assay method works properly strongly supports the hypothesis that insect LTP has a capacity to transfer and/or exchange lipids not only between the lipophorin particles with different densities but also between lipophorins with the same density.

There are two possible explanations for the process of lipid transport by lipophorin from the sites of loading lipids, e.g., fat body, midgut, or oenocyte, to the sites of unloading lipids, e.g., muscle, ovary, or cuticle. Lipophorin itself moving between two sites to load and unload lipids is one possibility. However, this seems unlikely, because, unlike vertebrates, insects have an open circulatory system without a definite bloodstream. The other, more likely, possibility is that lipids primarily taken up by lipophorin from the tissues are rapidly transferred and/or exchanged between lipophorin particles irrespective of the density by the action of LTP, which enables lipophorin to deliver the lipids from the loading sites to the unloading sites without lipophorin itself having to move between the two sites. This can be considered to be the physiological role of LTP in the process of lipid transport by lipophorin. Thus, if the loading rate of lipids by lipophorin equals the unloading rate, the lipid content would stay at practically the same level in all lipophorin particles, as can be seen in the case of flightless insects such as the cockroach or in resting locusts in which lipophorin exists in the hemolymph only as HDLp. When the loading rate exceeds the unloading rate, the lipid content would be equally elevated in all lipophorin particles, as observed for locusts injected with AKH in which all lipophorins are found as LDLp. Under opposite conditions, the lipid level in all lipophorin particles would decrease equally. This hypothesis is supported by the previous observation (13) that all lipophorin particles present in the hemolymph from a certain individual display homogeneity in density, although the density varies in accordance with the physiological conditions of insects from which the hemolymph has been collected.

Finally, we assume that LTP as well as lipophorin may be present in the hemolymph of almost all species of insects, and that all the functional aspects of American cockroach LTP observed in this study may be extended to LTP from other insects.

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