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Role of lipid transfer particle in delivery of diacylglycerol from midgut to lipophorin in larval *Manduca sexta*

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Abstract

The present work analyzed the function of lipid transfer particle (LTP) in the process of exporting diacylglycerol from larval *Manduca sexta* midgut cells to lipophorin. When midgut sacs, which had been prelabeled in vivo with [³H]oleic acid, were incubated in vitro with a lipophorin-containing medium, a significant amount of radiolabeled diacylglycerol was transferred to lipophorin. Negligible amounts of diacylglycerol were released into lipophorin-free medium. In contrast, lipid-labeled lipophorin did not transfer diacylglycerol to the midgut sacs. The transfer of diacylglycerol from the midgut sac to lipophorin was blocked by preincubation of midgut sacs with antibody against LTP. Diacylglycerol transfer was restored to control values by the addition of purified LTP to midgut sacs that had been treated with antibody against LTP. Under these conditions the amount of diacylglycerol transferred was a function of the LTP concentration. These are the first results showing that LTP is required to export diacylglycerol from the midgut to lipophorin. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: LTP; Lipophorin; HDLp; Insect; Hemolymph

1. Introduction

The tobacco hornworm *Manduca sexta* is widely used as a model of lipid transport in insects (Law and Wells, 1989; Law et al., 1992). During larval development, the main insect lipoprotein, lipophorin, cycles between the midgut, where it picks up diacylglycerol (DAG) produced from dietary lipids, and the fat body, where it delivers the DAG for storage as triacylglycerol (TAG) (Tsuchida and Wells, 1988; Soulages and Wells, 1994; Arrese et al., 2001). During the cycling, lipophorin works as a reusable shuttle without being accumulated or degraded in the tissues (Chino, 1985; Ryan, 1990; Van der Horst, 1990; Arrese et al., 2001). As a result of this process, feeding larvae accumulate TAG in the fat body, which can amount to nearly 30% of the wet weight of the tissue and can sustain the metabolic requirements

during the non-feeding periods of development (Law et al., 1992; Soulages and Wells, 1994).

We previously showed that dietary fatty acid (FA) is converted in the midgut to DAG via the phosphatidic acid pathway (Canavoso and Wells, 2000). We also showed that the midgut TAG pool serves as a reservoir from which DAG is produced at a rate consistent with its export into hemolymph lipophorin (Canavoso and Wells, 2000). However, the mechanism of DAG transfer from the midgut cells to lipophorin remained unknown. The protein responsible for specific binding of lipophorin to the midgut of larval *M. sexta* (Gondim and Wells, 2000) and lipid transfer particle (LTP) might be critical in the DAG transfer process.

LTP was first isolated from the hemolymph of *M. sexta* (Ryan et al., 1986a,b). It is a very high-density lipoprotein, which contains about 14% lipid and three apolipoproteins (apoLTP-I, -II and -III of 350, 85 and 60 kDa, respectively; Soulages and Wells, 1994; Ryan and Van der Horst, 2000; Arrese et al., 2001). The physiological role of LTP is not completely elucidated, but in vitro LTP catalyzes lipid exchange and net transfer between different lipophorins and between lipophorin

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and human lipoproteins by a carrier-mediated mechanism (Blacklock et al., 1992; Blacklock and Ryan, 1994; Ryan and Van der Horst, 2000). It was also hypothesized that LTP may function *in vivo* to catalyze lipid transfer between cells and lipophorin (Ryan et al., 1988), but there is little direct evidence to support this idea. Van Heusden and Law (1989) demonstrated *in vitro* that LTP promotes DAG transfer from fat body to lipophorin during hormone-induced mobilization of lipid stores. However, lipid transfer in the opposite direction was not mediated by LTP. In addition, LTP was shown to be involved in the transfer of lipids from lipophorin to developing oocytes (Liu and Ryan, 1991).

Lipid loading of lipophorin at the midgut is a critical event during absorption of dietary lipids. In this work, we have focused on the role of LTP in this process. Using an *in vitro* system, consisting of lipid-labeled midgut sacs and anti-LTP antibody, we show that release of DAG from midgut is an LTP-mediated process.

2. Materials and methods

2.1. Materials

[9,10-³H]Oleic acid was purchased from NEN (Boston, MA). Silica gel plates were obtained from J.T. Baker. AEBSF [4-(2-aminoethyl)-benzenesulfonylfluoride], trehalose and DEAE-trisacryl M were from Sigma (St. Louis, MO). Falcon multi-well tissue culture plates were obtained from Becton Dickinson (Franklin Lakes, NJ) and Affi-Gel Protein A from Bio-Rad (Hercules, CA). Centriprep Centrifugal Filter Devices were from Millipore-Amicon (Bedford, MA). All other chemicals were analytical grade.

2.2. Insects and hemolymph collection

M. sexta was reared as previously described (Prasad et al., 1986). Hemolymph from 2-day-old-fifth instar larvae was collected in ice-cold bleeding solution (30 mM KH₂PO₄ pH 6.5, containing 2 mM Na₂EDTA, 10 mM glutathione, and 3 mM NaN₃) by puncturing a proleg and gently pressing the abdomen. Hemolymph was centrifuged 5 min at 12 000g (4°C) to remove hemocytes.

2.3. Purification procedures

High-density lipophorin (Lp) and LTP were isolated from the hemolymph after two steps of KBr gradient, according to Shapiro et al. (1984) and Prasad et al. (1986). Briefly, in the first step 20 ml of hemocyte-free hemolymph were adjusted to a density 1.31 g/ml with KBr, transferred to a 39-ml Quick-Seal centrifuge tube and overlaid with 0.15 M NaCl. Centrifugation was carried out for 4 h at 50 000 rpm (4°C) in a Beckman

VTi 50 rotor and fractions corresponding to lipophorin (density 1.14 g/ml) and LTP (density 1.23 g/ml) were collected. In the second step, the lipophorin and LTP fractions were individually adjusted to 1.31 g/ml with KBr and 20-ml fractions of each preparation was overlaid with a 1.21 g/ml KBr solution in 0.15 M NaCl. Centrifugation was carried out for 16 h as described above. Lipophorin and LTP were then recovered from the top 5 ml of the gradient. Lipophorin was dialyzed against lepidopteran saline (5 mM KH₂PO₄, 100 mM KCl, 4 mM NaCl, 15mM MgCl₂, 2 mM CaCl₂, pH 6.5) and concentrated by ultrafiltration before use (Centriprep YM-50, Amicon).

After dialysis against 20 mM Tris-HCl, pH 8.7, containing 5 mM EDTA, LTP was purified before each experiment by a double passage through a DEAE-Trisacryl M column (Tsuchida et al., 1998). LTP was eluted with a linear 0–300 mM NaCl gradient (flow rate 15 ml/h) and 3 ml fractions were collected. LTP-containing fractions were pooled and stored at 4°C in 3.7 M KBr and 1 mM AEBSF for no longer than 1 week. Before its use, LTP was dialyzed against lepidopteran saline and concentrated as described above for lipophorin.

2.4. Lipoprotein-free hemolymph

The green-colored 9 ml at the bottom of the gradients from the first KBr gradients described above for the purification of lipophorin and LTP were collected. These samples were subjected to a second KBr gradient after adjusting the density to 1.31 g/ml KBr (final volume 20 ml) and overlaying with a 1.21 g/ml KBr solution in NaCl 0.15 M. Centrifugation was carried out for 16 h at 50 000 rpm (4°C) and all the green material (due to the presence of bile pigments associated with hemolymph proteins) localized about 16 ml from the top of the gradient was collected, but the very yellow bottom fraction was discarded. The sample was dialyzed against lepidopteran saline and concentrated by ultrafiltration (Centriprep YM-3, Amicon) to a concentration of about 90 mg/ml. One ml aliquots of the concentrated material were stored at –80°C. As determined by SDS-PAGE and immunoblotting assays using antibodies against apo-lipophorin-I and -II and LTP, this material was devoid of lipophorin and LTP. The material was termed “lipoprotein-free hemolymph” and was used in the midgut sac incubations at concentration of 25 mg/ml of incubation medium.

2.5. [³H]Lipid prelabeled-midgut sacs

To obtain midgut sacs with lipid pools labeled to constant specific activity with [³H]oleic acid, 2-day-old-fifth instar larvae were fed for 24 h with a [³H]oleic acid-containing artificial diet (4 µCi/g) (Canavoso and Wells, 2000). After feeding, insects were anesthetized with ice

and dissected under cold lepidopteran saline. The midgut was exposed, and without removing the contents, was rinsed with cold saline and the Malpighian tubules were removed carefully. The region corresponding to the midgut was ligated at both ends with surgical silk, the resulting midgut sac was extensively washed with cold lepidopteran saline and transferred to a 12-well culture plate containing 3 ml of incubation medium composed of modified lepidopteran saline (5 mM KH_2PO_4 , 60 mM KCl, 30 mM NaCl, 2 mM CaCl_2 , 30 mM MgCl_2 , 30 mM trehalose, 200 mM glucose, 100 mM sucrose, pH 6.5) containing 25 mg/ml of lipoprotein-free-hemolymph. After 5 min, the sac was transferred to a fresh medium and washed for a further 5 min. After this treatment, the labeled midgut sacs were ready to be employed in the incubations described below.

2.6. Midgut sac incubations

After the washing steps, [^3H]oleic acid prelabeled-midgut sacs were transferred to 3 ml of fresh medium and incubated with gentle shaking at room temperature with or without lipophorin (1 mg/ml). At different times, midgut sacs were removed, washed twice for 5 min each with 3 ml of incubation medium and the washes were combined with the medium. The combined washes and medium were then centrifuged (5 min at 2500g) to remove cellular debris, if present, and processed immediately for lipid extraction or subjected to a KBr gradient. Lipids were fractionated by thin-layer chromatography (TLC) and spots scraped from the gel assayed for radioactivity by liquid scintillation counting (Canavoso and Wells, 2000).

In another set of experiments, DAG transfer from labeled lipophorin to unlabeled midgut sacs was analyzed. Two-day-old-fifth instar larvae were fed [^3H]oleic acid in a small piece of artificial diet for one hour before bleeding. [^3H]DAG labeled lipophorin was isolated, as described above, from the hemolymph. About 95% of the label in lipophorin was DAG. Unlabeled midgut sacs were incubated with [^3H]DAG-Lp (1 mg/ml, sp. act.= 7.4×10^5 dpm/mg lipophorin) in lipoprotein-free hemolymph/lepidopteran saline incubation medium, as described above. At different times, midgut sacs were removed and washed twice with incubation medium containing unlabeled lipophorin (1 mg/ml) and twice with incubation medium. Radioactivity was analyzed in the combined washes and in midgut tissue, after removal of the luminal contents.

Lack of transfer of trypan blue from the lumen of the midgut sac to the incubation medium was used to assess tissue integrity and cellular viability.

2.7. Effect of LTP on lipid release from isolated midgut sacs

[^3H]Oleic acid prelabeled midgut sacs were transferred to fresh medium and incubated as described above

under the following conditions: (a) for 1 h in the presence of lipophorin (1 mg/ml); (b) for 30 min with anti-LTP antibody (3.5 mg IgG/ml), washed with medium and then transferred to medium with lipophorin (1 mg/ml) and incubated for 1 h; and (c) the same as in (b) and then transferred to a medium with both, lipophorin (1 mg/ml) and LTP (40 $\mu\text{g/ml}$). After incubation, midgut sacs were removed and washed twice for 5 min with 3 ml of incubation medium. The washes were combined with the medium and the material was centrifuged and processed immediately for lipid extraction or subjected to a KBr gradient, as described above.

To assess the effect of LTP concentration on the transfer of [^3H]DAG, labeled midgut sacs were preincubated with anti-LTP antibody, as described above, and then transferred to a medium containing lipophorin (1 mg/ml) and different concentrations of LTP. After incubation, the medium was processed as above.

2.8. Anti-LTP antibody

Antiserum against purified LTP was obtained from a New Zealand white rabbit as described by Ryan et al. (1988). The IgG fraction was then purified using Affigel Protein A (Bio-Rad) and stored at -80°C .

2.9. Protein and lipid determination

Protein concentration was determined by the Bradford assay (1976), using bovine serum albumin as standard. Lipids were extracted according to Folch et al. (1957) or Bligh and Dyer (1959). Lipid classes were separated by TLC on silica gel using hexane-ethyl ether-formic acid 70:30:3 v/v/v as a solvent system (Henderson and Tocher, 1992).

2.10. Statistical analysis

Statistical tests and nonlinear regression analysis were performed using GraphPad Prism version 3.00 for Windows (GraphPad Software, San Diego, CA). The results are expressed as a mean \pm SEM. $P < 0.05$ was considered as a significant difference between means.

3. Results and discussion

3.1. In vitro incubation system

A useful approach for investigating the role of LTP in the transfer of lipids between tissues and lipophorin is the use of anti-LTP antibody to inhibit lipid delivery in an in vitro assay system. This was the rationale by which Van Heusden and Law (1989) demonstrated that LTP promotes DAG transfer from fat body to lipophorin in adult *M. sexta*. In addition, Liu and Ryan (1991) dem-

onstrated that anti-LTP antibody inhibited lipid transfer to oocytes *in vitro*.

However, the development of an *in vitro* system for incubating insect tissue that mimics *in vivo* conditions is frequently difficult. Often the release of DAG into the incubation medium occurs to only a limited extent (Chino et al., 1989; Arrese et al., 1996). Therefore, in order to elucidate the role of LTP in the transfer of lipids from the midgut of *M. sexta* larvae to lipophorin, we first had to develop an *in vitro* system that supported reliable DAG transfer from [³H]oleic acid-labeled midgut sacs.

Bauerfeind and Komnick (1992) employed labeled midgut sacs and whole hemolymph for biochemical and immunocytochemical studies of lipid loading and unloading of lipophorin at the midgut of the dragonfly, *Aeshna cyanea*. In *Rhodnius prolixus*, studies of loading of lipophorin with phospholipids at the midgut were also performed using ³²P-labeled posterior midgut sacs (Atella et al., 1995). Midgut sacs of *M. sexta* larvae were previously employed by Tsuchida and Wells (1988) in studies of lipid digestion and absorption. In that study, ³H-labeled triolein was injected into the lumen of the sacs, which were incubated for 30 min in Graces' medium. However, after several experiments, we concluded that those conditions were not optimal for our studies because: (1) Graces' medium was unsuitable for maintaining the integrity of the midgut sacs for >30 min, as determined by leakage of trypan blue from the lumen of the sac into the incubation medium; (2) the microinjection of lipids into the lumen of the sacs often resulted in leakage of Trypan Blue; and (3) even when no leaks were observed, microinjecting midgut sacs with either radioactive FA or triolein often did not lead to efficient transfer of [³H]DAG to lipophorin in the medium. The use of lipoprotein-free hemolymph in the incubation medium and pre-labeled midgut sacs overcame all of these problems.

3.2. DAG transfer from midgut to lipophorin *in vitro*

We previously demonstrated that midgut lipid pools could be homogeneously labeled by feeding insects with [³H]oleic acid for 24 h (Canavoso and Wells, 2000). As shown in Fig. 1, when [³H]oleic acid-labeled midgut sacs were incubated with lipoprotein-free hemolymph/lepidopteran saline and lipophorin there was significant transfer of radioactive lipids into the medium. In contrast, when labeled midgut sacs were incubated in the absence of lipophorin, a negligible amount of radioactivity was released to the medium at any time (Fig. 1). In the presence of lipophorin >80% of the total radioactivity recovered in the medium was [³H]DAG (Fig. 1, inset). In the absence of lipophorin, about 20% of the recovered radioactivity was found in DAG, 33% in FA, 22% in PL with traces in mono- and triacylgly-

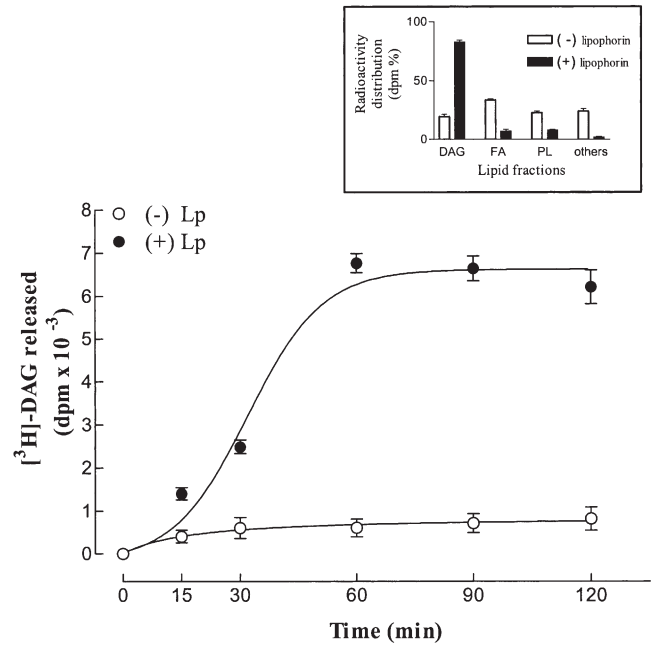


Fig. 1. Time-course of [³H]DAG transfer *in vitro* from midgut sacs. [³H]Lipid pre-labeled midgut sacs were incubated without (—○—) or with (—●—) lipophorin (1 mg/ml incubation medium, final volume 3.0 ml). At various times, midgut sacs were removed and the media processed for lipid extraction. Extracted lipids were separated by TLC and the radioactivity determined by liquid scintillation counting of silica gel scrapings. Results are expressed as total dpm±SEM (*n*=8) in DAG fraction. The inset shows the distribution of radioactivity among lipids from the medium (without and with lipophorin) after 2-h incubation. The results, representative of the distribution at other times analyzed, are expressed as a percentage of dpm±SEM. The sum of dpm found in DAG, FA, PL and others (mono- and triacylglycerol) represents 100%.

cerol (Fig. 1, inset). These observations showed that the [³H]DAG recovered in the medium was due to loading of lipophorin with lipids from the labeled midgut sacs.

The fact that the export of DAG from the midgut sacs depended on the presence of lipophorin is in agreement with the role of lipophorin as the DAG carrier in insect hemolymph and with previous *in vitro* observations in *M. sexta* (Tsuchida and Wells, 1988) and *A. cyanea* (Bauerfeind and Komnick, 1992). The release of phospholipids from labeled midgut sacs was also dependent on the presence of lipophorin in the incubation medium (Atella et al., 1995). The small amount of labeled FA found in the medium, with or without lipophorin, indicated the absence of leaks in the isolated sacs, because the luminal contents of the midgut sacs contained large amounts of radioactive FA from the diet. Longer incubations, previously reported unsuitable for midgut preparations (Rana and Stanley, 1999), induced loss of tissue integrity, as judged by leakage of Trypan Blue.

These data show that under our experimental conditions isolated midgut sacs from larval insects were competent to export DAG to lipophorin in the medium for 2 h. Most of the export occurred during the first hour,

with less export occurring later, perhaps reflecting saturation of the lipophorin with DAG. The midgut sacs mimic the physiological properties of the midgut in exporting dietary lipids to the lipophorin (Canavoso and Wells, 2000).

3.3. Transfer of DAG from lipophorin to midgut in vitro

When midgut sacs were incubated with [^3H]DAG labeled lipophorin, no significant amount of the label was transferred to the tissue. Over the 2 h incubation, the amount of label transfer was $0.26\% \pm 0.18$ and did not vary significantly with time, and the amount of radiolabeled lipids recovered from the tissue was too small to analyze. These data showed that transfer of lipid from midgut to lipophorin was unidirectional, at least under feeding conditions. These data are consistent with the observation that injection of [^3H]DAG-lipophorin into feeding *M. sexta* larvae lead primarily to labeling of the fat body as TAG (Tsuchida and Wells, 1988; Canavoso and Wells, unpublished). In *A. cyanea* (Bauerfeind and Komnick, 1992) and *R. prolixus* (Atella et al., 2000), DAG and FA transfer, respectively, were reversed in fasting midguts. We did not perform any experiments with fasting larval *M. sexta*.

3.4. Role of LTP in DAG transfer from midgut to lipophorin in vitro

When [^3H]oleic acid-labeled midgut sacs were preincubated with anti-LTP antibody for 30 min and then transferred to a medium with lipophorin, the amount of [^3H]DAG released into the medium was significantly reduced (Fig. 2B). On the other hand, labeled midgut sacs preincubated with non-immune rabbit serum and then transferred to a medium with lipophorin, released [^3H]DAG into the medium to the same extent as control midgut sacs (Fig. 2A). These results showed that the inhibitory effect of anti-LTP antibody on lipid release from the midgut was specific and not an artifact produced by immune serum proteins. When [^3H]labeled midgut sacs were preincubated with anti-LTP antibody and then transferred to a medium with both lipophorin and LTP, the amount of lipid released into the medium was restored to control values (Fig. 2B).

When midgut sacs treated with anti-LTP antibody were incubated with lipophorin and increasing concentrations of LTP, the amount of [^3H]DAG transferred to lipophorin increased with increasing concentrations of LTP (Fig. 3). Nonlinear regression analysis showed that half-maximal activation occurred at an LTP concentration of $37 \pm 10 \mu\text{g/ml}$. The amount of LTP necessary to promote in vitro lipid release may be of physiological relevance because we used a concentration of lipophorin that is close to the physiological levels [$\sim 3 \text{ mg/animal}$

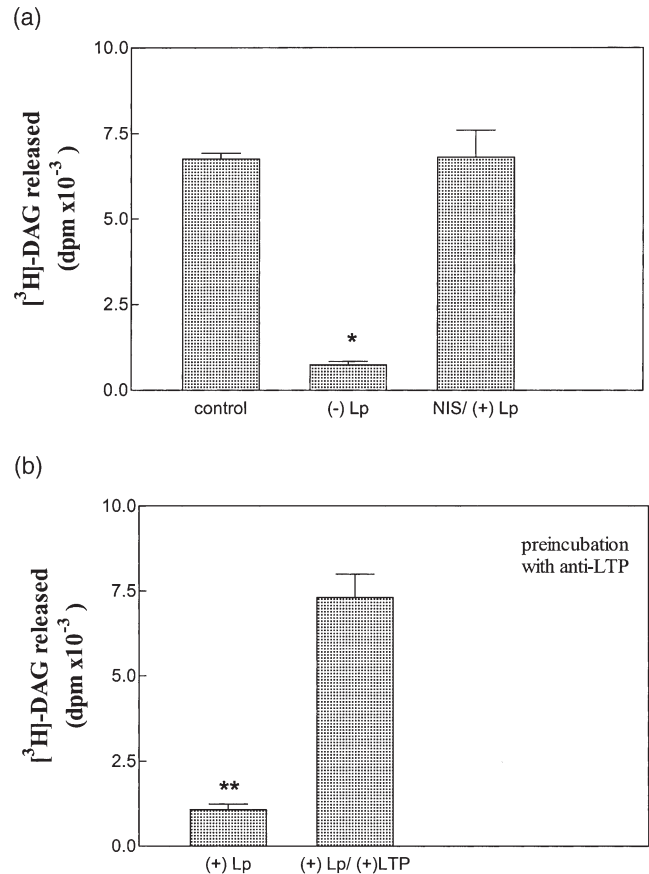


Fig. 2. Effect of LTP on [^3H]DAG transfer in vitro from labeled midgut sacs. [^3H]Prelabeled midgut sacs were transferred to a medium with lipoprotein-free hemolymph/modified lepidopteran saline and incubated as described in Materials and Methods under the following conditions: (A) in the presence of lipophorin (1 mg/ml) [control]; without lipophorin, [(-) Lp]; or preincubated with rabbit non-immune serum (NIS, 0.5 ml) and then transferred to a medium with lipophorin (1 mg/ml) [NIS/(+) Lp]. (B) Preincubated with anti-LTP antibody (3.5 $\mu\text{g IgG}/\mu\text{l}$) and then transferred to a medium with lipophorin, [(+) Lp] or with both, lipophorin (1 mg/ml) and LTP (40 $\mu\text{g}/\text{ml}$), [(+) Lp/(+) LTP]. Concentrations for lipophorin and LTP are expressed as mg or μg per ml of incubation medium (3 ml final volume). After incubation, midgut sacs were removed and the media processed for lipid extraction as previously stated. Lipids were fractionated by TLC and spots assayed for radioactivity by liquid scintillation counting. Results are expressed as total dpm \pm SEM ($n=6-8$) found in DAG fraction. * $P < 0.001$ versus control and NIS/(+) Lp. ** $P < 0.001$ versus control, NIS/(+) Lp and (+) Lp/(+) LTP.

for second-day fifth instar larvae, Prasad et al. (1987)] and the hemolymph level of LTP ranges between 50 and 80 $\mu\text{g/ml}$ during the fifth instar (Van Heusden et al., 1996).

The fact that midgut sacs exported [^3H]DAG to lipophorin in the absence of exogenous LTP (Fig. 1 and controls in Figs. 2 and 4) suggests that LTP is bound to the isolated midgut. This midgut membrane-bound LTP is sufficient to facilitate DAG transfer and cannot be eliminated even after extensive washing, as is also the case in fat body (Van Heusden et al., 1996). Van Heusden

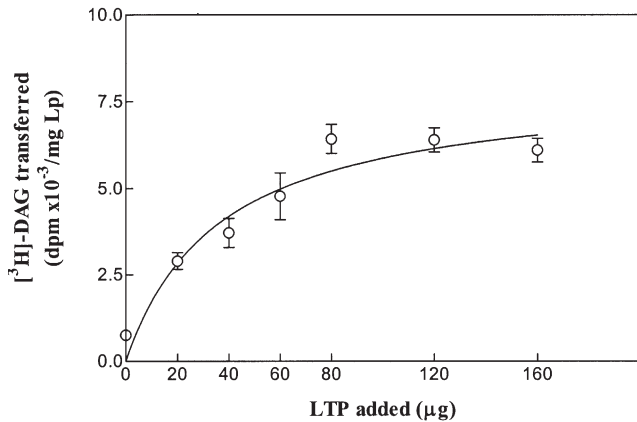


Fig. 3. Effect of LTP concentration on the transfer of [³H]DAG. ³H-labeled midgut sacs were incubated with anti LTP (3.5 µg IgG /µl) as described under Material and Methods and then, transferred to a new medium containing lipophorin (1 mg/ml) and different amounts of LTP. After 1-h incubation, media were processed for lipid extraction. Lipids were fractionated by TLC and the spot corresponding to DAG scraped from the gel and assayed for radioactivity by liquid scintillation counting. Labeled DAG was associated with lipophorin and therefore, results are expressed as total dpm in [³H]DAG per mg of lipophorin.

and Law (1989) found that transfer of lipids from labeled fat bodies in adult *M. sexta* to lipophorin also occurred in the absence of added LTP and suggested that intrinsic LTP from fat body tissue was responsible for the transfer. It is not known whether this LTP arose from synthesis by the fat body (Van Heusden et al., 1996) or from the binding of LTP from hemolymph. Liu and Ryan (1991) reported that LTP was involved in the transformation of lipophorin within *M. sexta* oocytes, although it is not clear if the oocyte LTP was the result of *de novo* synthesis by the follicle cells or uptake from hemolymph. It is also unknown whether the midgut can make LTP.

These results also imply that LTP catalyzes a net DAG transfer between midgut sacs and lipophorin, because we showed that DAG transfer from lipophorin to midgut sacs does not occur.

3.5. Characterization of lipids bound to lipophorin

When the incubation medium from control experiments was analyzed in a KBr gradient, the radioactivity was associated with lipophorin (Fig. 4A). When the incubation was carried out with midgut sacs, which were previously incubated with anti-LTP antibody, there was negligible radioactivity associated with lipophorin (Fig. 4B). But when the incubation was carried out with anti-LTP antibody and then purified LTP was added into the medium, the radioactivity again associated with lipophorin (Fig. 4C). In all three cases, about 80–85% of the radioactivity recovered in lipophorin was found in the DAG fraction, with 10–15% in FA, 2–4.6% in PL and

traces of radioactivity in MAG and TAG (~0.7%) (Fig. 4D–F). Note that although the distribution of radioactivity did not change, the amount of radioactivity found in lipophorin when anti-LTP antibody was present was very small.

3.6. Summary

The results presented here show for the first time that LTP is a necessary factor in exporting DAG from the midgut to lipophorin. We also showed that LTP catalyzes unidirectional transfer of DAG from the midgut to lipophorin. Although experimental evidence indicates that LTP can facilitate lipid transfer between lipophorins via a carrier-mediated mechanism (Blacklock et al., 1992; Blacklock and Ryan, 1994; Ryan and Van der Horst, 2000), little is known about the molecular aspects of lipid transfer between tissues and lipophorin. For the LTP lipid-mediated transfer process between fat body and lipophorin, a hypothetical model suggested by the morphological asymmetry of LTP has been discussed (Blacklock and Ryan, 1994), but there is no evidence to support this model. Arrese et al. (2001) postulated a possible lipid transfer complex in which LTP is proposed to mediate the transfer of any lipid with specificity being determined by the properties of putative lipid transfer factors in the cell membrane. Here we have provided some experimental support for that model by showing that LTP is necessary for DAG export from the midgut. We also showed that midgut tissue contains enough LTP bound to promote DAG release. Specific lipophorin binding to the midgut membranes was recently characterized (Gondim and Wells, 2000) and it is possible that LTP works in concert with a midgut lipophorin receptor to promote lipid release. Clearly, a better characterization of the midgut lipophorin receptor and the identification of a DAG specific transfer factor in midgut membrane are necessary to truly elucidate the mechanism by which LTP mediates lipid transfer from midgut cells to lipophorin.

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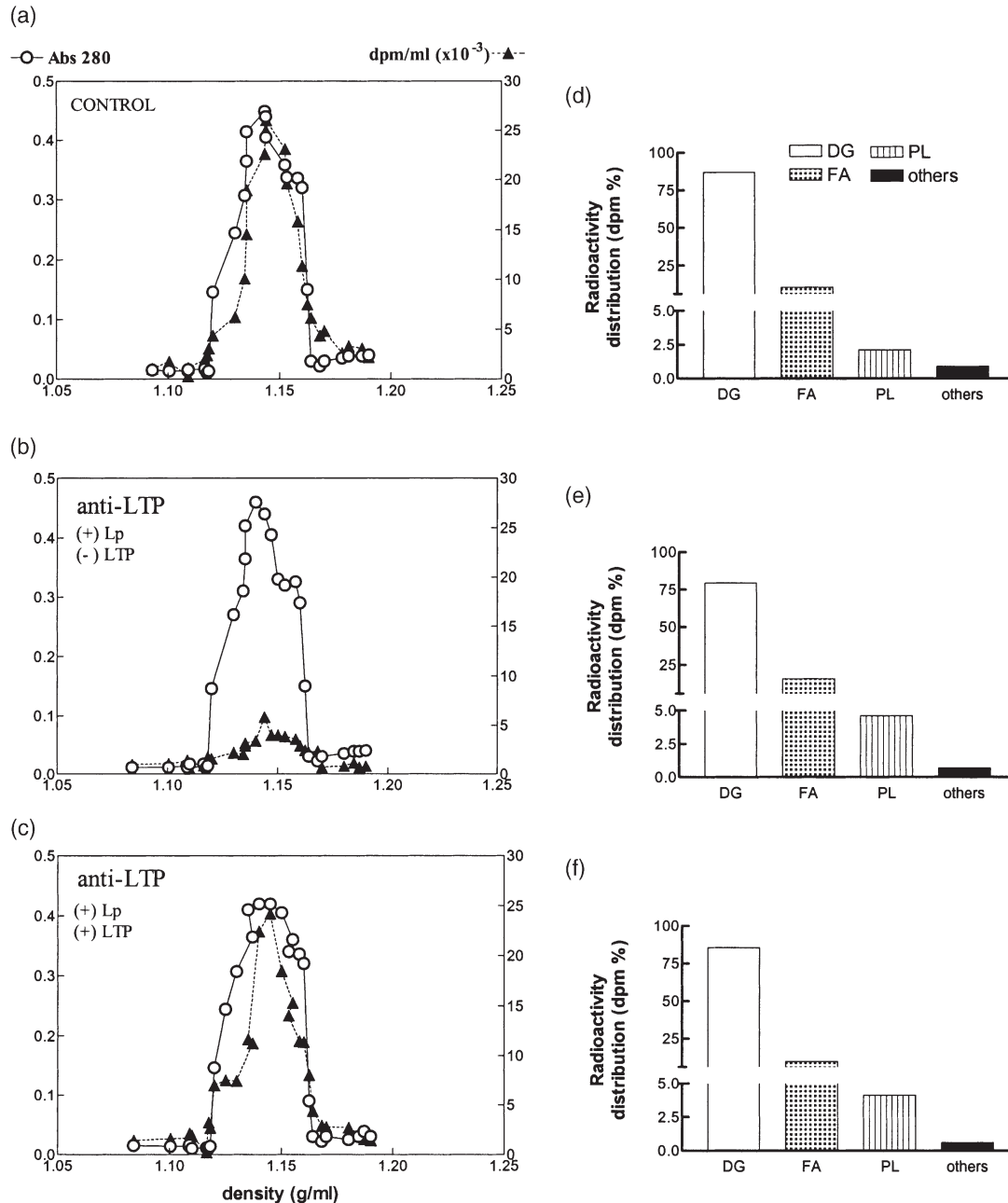


Fig. 4. Lipid transfer from prelabeled midgut sacs to lipophorin—characterization of lipids bound to lipophorin. Density gradient ultracentrifugation profiles of incubation media ($n=3$) after *in vitro* incubation of ³H-prelabeled midgut sacs as follows: (A) with lipophorin (1 mg/ml) [control]; (B) preincubated with anti-LTP (3.5 μ g IgG/ μ l, 30 min at room temperature) and then transferred to a medium containing lipophorin (1 mg/ml), [(+) Lp/(-) LTP] and (C) same as (B) but transferred to a medium with lipophorin (1 mg/ml) and LTP (40 μ g/ml) [(+) Lp/(+) LTP]. After centrifugation, the tubes were fractionated from the top into 1-ml fractions and density, absorbance (\circ -) and radioactivity (\blacktriangle -) in each fraction determined. Panels D–F shows the distribution of radiolabel among lipophorin lipids for each incubation condition, respectively. Lipids from lipophorin were extracted and separated by TLC. Lipid spots were scraped from the gel and assayed for radioactivity by liquid scintillation counting. Results are expressed as a percentage of dpm, where the sum of dpm found in DAG, FA, PL and others (mono- and triacylglycerol) represents 100%.

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