Lipophorin structure analyzed by in vitro treatment with lipases

John K. Kawooya,[1,*] Dick J. van der Horst,[†] Miranda C. van Heusden,[2,*] Bernadette L. J. Brigot,[*] Rik van Antwerpen,[*] and John H. Law[*]

Department of Biochemistry and Center for Insect Science,[*] Biological Sciences West, University of Arizona, Tucson, AZ 85721, and Department of Experimental Zoology,[†] University of Utrecht, Padualaan 8, 3584 CH Utrecht, The Netherlands

Abstract Adult *Manduca sexta* high density lipophorin (HDLp-A) is composed of three apolipoproteins (apoLp-I, -II, and -III) and 52% lipid. The flight-specific low density lipophorin (LDLp) contains 62% lipid and is associated with several additional molecules of apoLp-III. The amount of phospholipid remains constant in lipophorin (140 mol/mol of lipophorin), while the diacylglycerol content varies between different lipophorin species (310 mol/mol HDLp up to 1160 mol/mol LDLp). Both lipophorin particles were enzymatically depleted of phospholipid or diacylglycerol by in vitro incubation with either phospholipase A_2 or triacylglycerol lipase. Albumin was used to remove free fatty acids generated during the reaction. Treatment with phospholipase A_2 removed all phospholipids (except sphingomyelin) and the resulting particles were stable. Triacylglycerol lipase hydrolyzed large fractions of diacylglycerol. The resulting particles were smaller in size, higher in density, and devoid of apoLp-III. The particles retained apoLp-I and -II and the other lipid components, including a substantial amount of diacylglycerol. Structural integrity of diacylglycerol-depleted lipophorin was confirmed by electron microscopical analysis. When treated with both phospholipase A_2 and triacylglycerol lipase, lipophorin precipitated. From these results we conclude that: *1*) all phospholipid and apoLp-III are located at the surface of lipophorin, whereas diacylglycerol is partitioned between the sublayers and the surface of the particle; *2*) both diacylglycerol and phospholipid play a role in stabilizing lipophorin in the aqueous medium; and *3*) lipophorin can be extensively unloaded and still retain its basic structure, a necessary feature for its function as a reusable lipid shuttle.—**Kawooya, J. K., D. J. van der Horst, M. C. van Heusden, B. L. J. Brigot, R. van Antwerpen, and J. H. Law.** Lipophorin structure analyzed by in vitro treatment with lipases. *J. Lipid Res.* 1991. **32:** 1781–1788.

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In insect hemolymph, lipids are transported by lipophorin, the major insect lipid-carrying protein (1–5). Lipophorin of the adult *M. sexta* is a high density lipoprotein consisting of three apolipoproteins (apolipophorin I, apoLp-I, $M_r \approx 2.5 \times 10^5$, apolipophorin II, apoLp-II, $M_r \approx 8.0 \times 10^4$, and apolipophorin III, apoLp-III, $M_r \approx 1.7 \times 10^4$) combined with lipids, mainly diacylglycerol (DAG) and phospholipid (PL) (6). Lipophorin metabolism has been studied most extensively in hemolymph of adult *Locusta migratoria* and *M. sexta* during flight or after injection with adipokinetic hormone (AKH) (7–11). In adult *M. sexta,* the effects of AKH (12) culminate in the conversion of the high density lipophorin (HDLp-A, $M_r \approx 7.63 \times 10^5$; d ≈ 1.076 g/ml) to the DAG-enriched, low density lipophorin (LDLp, $M_r \approx 1.56 \times 10^6$, d ≈ 1.030 g/ml) (13). The expanded hydrophobic surface of LDLp is stabilized in the aqueous phase of the hemolymph by several molecules of the third small apolipoprotein, apoLp-III (11, 14). The association of apoLp-III with lipophorin is reversible upon decreasing the DAG content of the particle.

Insect lipophorin can load and unload neutral lipids without destruction of the particle and was proposed to function as a reusable lipid shuttle (15, 16). Although the molecular organization of insect lipophorin is not known, evidence from previous studies (17–19) suggests that it parallels that of mammalian lipoproteins (20–23). The proposed model for insect lipophorin consists of a hydrophobic core containing DAG, hydrocarbon, TAG, and sterol, which is separated from the aqueous phase by apoLp-I, apoLp-II, and a mixed monomolecular film of PL, DAG, and, in some cases, apoLp-III (3, 18, 24).

Abbreviations: AKH, adipokinetic hormone; LDLp, low density lipophorin; HDLp, high density lipophorin; HDLp-A, adult high density lipophorin; VHDLp, very high density lipophorin; Lp, lipophorin; apoLp-I, apolipophorin I; apoLp-II, apolipophorin II; apoLp-III, apolipophorin III; MAG, monoacylglycerol; DAG, diacylglycerol; TAG, triacylglycerol; HC, hydrocarbon; PL, phospholipid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; FFA, free fatty acid; SP, sphingomyelin; PLA_2, phospholipase A_2; TAG-lipase, triacylglycerol lipase; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

[1]Present address: The Upjohn Company, 7000 Portage Road, Kalamazoo, MI 49001.

[2]To whom correspondence should be addressed.

The purpose of this study was to approach the physiological unloading of lipids from lipophorin (25–27) by establishing an in vitro system using lipolytic enzymes. With this model system, a series of particles with different lipid load were obtained, which were used to study structural aspects of lipophorin.

EXPERIMENTAL PROCEDURES

Materials

M. sexta were raised from eggs according to the method previously described (6). Materials were purchased from the following sources: BCA protein assay kit, Pierce, Rockford, IL; SDS-PAGE protein standards and Coomassie brilliant blue G-250, Bio-Rad Laboratories, Richmond, CA. *Crotalus durissus* phospholipase A_2 (PL-A_2) and *Candida cylindracea* triacylglycerol lipase (TAG-lipase) were from Boehringer Mannheim Biochemicals, Indianapolis, IN. Fatty acid-free bovine serum albumin, and lipid standards for thin-layer chromatography were from Sigma Chemical Company, St. Louis, MO. Radioactive orthophosphate and glycerol were from Amersham Corporation, Arlington Heights, IL. Omnifluor scintillant was from New England Nuclear, Boston, MA. Synthetic *M. sexta* adipokinetic hormone was from Peninsula Laboratories, Inc., San Carlos, CA. PD10 desalting column was from Pharmacia/LKB Biotechnology, Inc., Pleasant Hill, CA. Ultrogel AcA22 was from IBF Biotechniques, Savage, MD. Thiophilic T-gel was generously provided by Dr. J. Porath (University of Arizona).

General methods

Electrophoresis under reducing conditions (SDS-PAGE) (28) was performed on 4–15% acrylamide gradient slab gels. Gels were stained with Coomassie brilliant blue G-250 (0.6% in methanol–acetic acid–water 5:1:4). Protein was measured colorimetrically (29). The apparent (non-equilibrium) densities of lipophorins, after isolation, were measured by refractometry.

Isolation of hemolymph lipophorins

Both HDLp-A and LDLp were isolated from hemolymph of adult moths (1–2 days after emerging) by density gradient ultracentrifugation in KBr (30). Prior to isolation, LDLp was induced in hemolymph by injecting adipokinetic hormone (10 pmol/animal). One h later hemolymph was collected by the flushing-out method (31) in phosphate-buffered saline (0.1 M sodium phosphate, pH 7.0, containing 0.15 M NaCl, 10 mM EDTA, 10 mM glutathione, 1 mM diisopropyl fluorophosphate).

Radiolabeling of lipophorin

LDLp was radiolabeled in the PL fraction with ^{32}P and in the DAG moiety with [1(3)-^{3}H]glycerol. Moths (12–14 h after emerging) were injected with [^{32}P]orthophosphate (sp act 1 mCi/ml, 16 μCi/animal). After 24 h, the $^{32}PO_4$-labeled lipophorin was isolated from hemolymph as described above. LDLp was radiolabeled in the DAG fraction by injection with [1(3)-^{3}H]glycerol (sp act 2.9 Ci/mmole, 25 μCi/animal). After 1 h, AKH was injected (10 pmol/animal), and 1 h later, LDLp was isolated from hemolymph as described above. In either case, unincorporated radiolabel was removed by extensive dialysis of lipophorin solutions against 10 mM TES-buffer, pH 7.2, that contained 0.3 M NaCl and 7 mM $CaCl_2$. Nonradiolabeled lipophorin was also dialyzed against the above buffer to remove any traces of EDTA used during the collection of hemolymph.

Hydrolysis of lipophorin-bound PL and DAG

Prior to hydrolysis, defined amounts of lipophorin were incubated with 200 mg of fatty acid-free albumin in 2 ml TES-buffer containing protease inhibitors (1 mM phenylmethane sulfonylfluoride and 2 mM diisopropyl fluorophosphate) for 10 min at 34°C in a shaking water bath. After this period, either PLA_2 (sp act 200 units/mg; 25 μg of PLA_2 per 6 mg of lipophorin) or TAG-lipase (sp act 581 units/mg; amount added varied, see Results section) were added to the medium and the incubation was extended for a period of 3 h, except where indicated. The reaction was terminated by adding 50 μl of a 10 mM EDTA solution. Control incubations contained all the ingredients with the exception of the enzyme. At the end of incubation, samples were drawn from the mixture for lipid and protein analyses.

With the exception of the experiments described in Table 2, all incubations were performed in the presence of an excess of PLA_2 and/or TAG-lipase. Under the reaction conditions used, albumin (as a fatty acid acceptor) was the rate-limiting factor. Therefore, the extent of lipid removal and thus the density of the resulting lipophorin particle was dependent on the amount of albumin present in the medium which, due to its solubility, was limited to 200 mg/2 ml incubation mixture.

Isolation of lipophorins from the reaction mixture

After incubation, the reaction media were subjected to density gradient ultracentrifugation as described above. Density gradients were fractionated and fractions that contained lipophorin (as determined by SDS-PAGE) were pooled and equilibrated with 0.1 M Na-phosphate buffer that contained 0.6 M K_2SO_4, pH 7.4, using a PD10-desalting column. The lipophorin sample was then applied to a (1 × 15 cm) thiophilic T-gel (32) equilibrated with the same phosphate buffer. After all the unbound protein (serum albumin) was washed off the column, the bound lipophorin was eluted with 0.1 M Na-phosphate buffer, pH 7.4 (in the absence of K_2SO_4). Purity of lipophorin was analyzed by SDS-PAGE.

Gel permeation chromatography

Lipophorins were analyzed by gel permeation chromatography on an Ultrogel AcA22 (0.9 × 210 cm) column. The column was equilibrated with the TES-buffer, and was calibrated with lipophorins of known sizes (33).

Lipid analysis

Lipids were extracted from lipophorins (34) and were separated by thin-layer chromatography. Neutral lipids were separated in hexane–ether–acetic acid 60:40:1. The polar lipids were separated either by one-dimensional chromatography using dichloromethane–methanol–water 65:25:1 or by two-dimensional chromatography (35). Lipids were visualized with iodine vapor. After chromatography, the individual neutral lipids were quantitated by gas chromatography (36). PL from the two-dimensional thin-layer chromatographs were quantitated colorimetrically (37). Radiolabeled lipids were scanned with a thin-layer chromatography linear isotope analyzer (Model LB 238, Berthold).

Electron microscopy

Lipophorin particles were negatively stained with 1% uranyl acetate (38), and analyzed using a Philips EM 420 electron microscope at 60 kV.

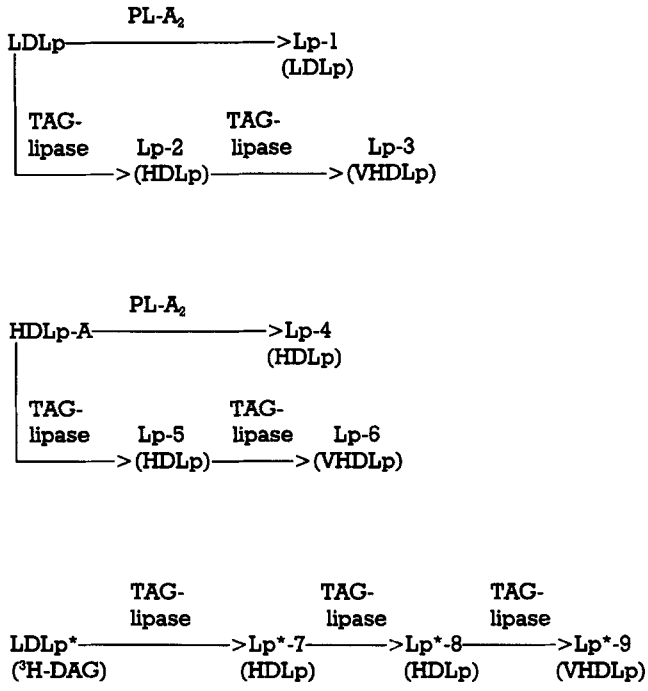

Fig. 1. Flow chart depicting how the lipid-depleted lipophorins were prepared by treatment with lipolytic enzymes.

RESULTS

Several modified forms of lipophorin were produced by treatment with lipolytic enzymes. They belonged to each of the three density classes: low density, high density, and very high density. **Fig. 1** shows a flow chart depicting the production of these lipid-depleted lipophorins.

Effect of PLA_2 on LDLp and HDLp-A

In order to determine whether lysophospholipids and free fatty acids play any role in lipophorin structure, we extracted these components by incubating native LDLp or HDLp-A with albumin. Albumin was able to extract LPC, LPE, and FFA without disrupting lipophorin structure. When LDLp was incubated with albumin in the presence of PLA_2, all PL (with the exception of SP) was eliminated from the particle (**Table 1**). The PLA_2-treated particle (Lp-1) was soluble in buffer. In a parallel experiment, incubation of HDLp-A with a mixture of PLA_2 and albumin also resulted in a PL-depleted particle (Lp-4), which was soluble in buffer (Table 1).

Effect of TAG-lipase on LDLp and HDLp-A

Fig. 2 shows the distribution of lipophorin and albumin (along a KBr gradient) after incubation of these two components with or without TAG-lipase. When incubated with albumin only, LDLp floated at a density range of 1.043–1.112 g/ml (Fig. 2A), where it was readily separated from albumin. However, when TAG-lipase was included in the medium, the density of LDLp increased while some of the albumin shifted to the lower density range of the gradient (Fig. 2B), presumably because it was saturated with fatty acids. An additional thiophilic affinity chromatographic step was necessary to separate the product lipophorin from the fatty acid-saturated albumin. Between 85 and 90% of lipophorin was recovered after incubation and subsequent purification.

Formation of Lp-2 (Table 1) was the end-point of lipase-catalyzed removal of DAG from LDLp under the reaction conditions used, since addition of excess enzyme (≥130 units/mg of LDLp) did not result in further removal of DAG. When Lp-2 was isolated and incubated with TAG-lipase and fresh albumin, more DAG and TAG were removed from Lp-2 to form a very high density lipophorin designated Lp-3 (Table 1). Although Lp-3 was buffer-soluble, the particle had a tendency to aggregate at protein concentrations of ≥1 mg/ml.

The effect of TAG-lipase on HDLp-A (Table 1) was very similar to that on LDLp. Purification of the modified particle (Lp-5) by density gradient ultracentrifugation and thiophilic affinity chromatography is summarized in **Fig. 3.** Incubation of Lp-5 with TAG-lipase resulted in a very high density particle, Lp-6, that was very similar to Lp-3 (Table 1).

TABLE 1. Properties of LDLp and HDLp-A before and after treatment with PLA_2 or TAG-lipase

	LDLp	Lp-1	Lp-2	Lp-3	HDLp-A	Lp-4	Lp-5	Lp-6
Protein (% weight)	38	44	55	76	48	64	67	75
Lipid (% weight)	62	56	45	24	52	36	33	25
PL[a]	141	16	138	140	141	14	142	139
DAG[a]	1162	1160	232	25	308	310	165	23
Density (g/ml)	1.052	1.058	1.130	1.265	1.109	1.118	1.187	1.264
ApoLp-I:ApoLp-II: ApoLp-III[b]	1:1:16	1:1:16	1:1:0	1:1:0	1:1:2	1:1:2	1:1:0	1:1:0
Radius[c] (Å)	84	84	65	51	67	67	55	52
Radius[d] (Å)	82	80	62	51	63	61	59	51
Surface area ($Å^2 \times 10^4$)	8.45	8.04	4.83	3.27	5.00	4.68	4.38	3.27
Volume ($Å^3 \times 10^{-6}$)	2.31	2.14	1.00	0.56	1.05	0.95	0.86	0.56

Lipophorin particles were incubated, in the presence of albumin (200 mg), with lipases at the following concentrations: Lp-1: 6 mg of LDLp with 25 μg PlA_2; Lp-2: 10 mg LDLp with 500 μg TAG-lipase; Lp-3: 8.6 mg Lp-2 with 430 μg TAG-lipase; Lp-4: 6 mg HDLp-A with 25 μg PLA_2; Lp-5: 9.5 mg HDLp-A with 500 μg TAG-lipase; Lp-6: 7.8 mg Lp-5 with 410 μg TAG-lipase. The resulting particles were isolated from the incubation media and analyzed as described under Experimental Procedures. Values represent the mean of three determinations. Standard deviations were ≤5% for lipid determinations, and between 1% and 7% for radius determinations. Density determinations varied less than 0.004 g/ml.

[a]Expressed as moles of lipid/mole of particle.

[b]Stoichiometry of apolipoproteins.

[c]From gel filtration.

[d]Calculated from chemical composition.

Effect of a PLA_2 and TAG-lipase mixture on modified very high density lipophorins

When modified very high density lipophorins, Lp-3 and Lp-6, were incubated with a mixture of PLA_2 and TAG-lipase, a precipitate was observed in the incubation mixture. After isolation and extensive washing, the precipitate was found (by SDS-PAGE) to contain apoLp-I and apoLp-II. The buffer-insoluble lipophorin was devoid of PL (except SP), DAG, MAG, and TAG, but contained 46 mol of hydrocarbon and 13 mol of sterol per mol of lipophorin. In a parallel experiment, a single incubation of native LDLp or HDLp-A with a mixture of PLA_2 and TAG-lipase resulted in modified buffer-soluble lipophorins. The densities, sizes, and neutral lipid compositions of these particles were similar to those of the corresponding TAG-lipase-treated particles Lp-2 and Lp-5 (Table 1), except that these particles were devoid of PL (other than SP).

Electron microscopic preparations of native and lipase-treated lipophorin

The morphologies of LDLp and HDLp-A were examined before and after the particles were treated with TAG-

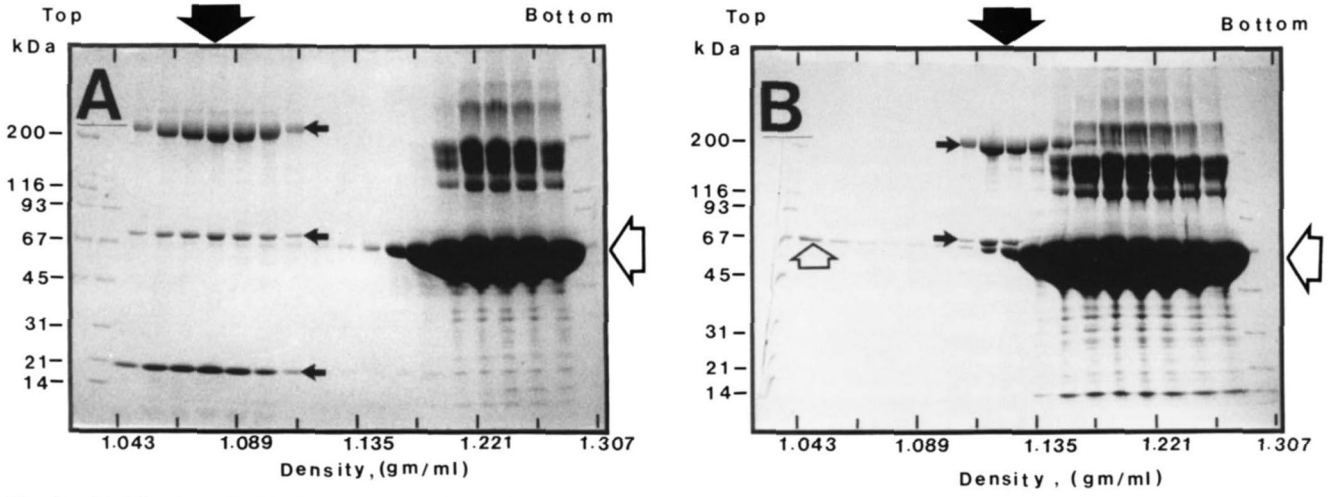

Fig. 2. Modification of LDLp by treatment with TAG-lipase. LDLp (10 mg) was incubated with 200 mg of fatty acid-free albumin in the absence (A) and the presence (B) of 1 mg TAG-lipase. After incubation (3 h, 34°C), the medium was subjected to density gradient ultracentrifugation as described under Experimental Procedures. (A) LDLp incubated with albumin. Large closed arrow: LDLp (d ≈ 1.058 g/ml). Small closed arrows: the three apolipoproteins of LDLp (from top to bottom; apoLp-I, $M_r \approx 2.5 \times 10^5$; apoLp-II, $M_r \approx 8.0 \times 10^4$; apoLp-III ≈ 1.7×10^4). Large open arrow: albumin. (B) LDLp incubated with albumin in the presence of TAG-lipase. Large closed arrow: Lp-2 (d ≈ 1.133 g/ml). Small closed arrows: the two apolipoproteins of Lp-2 (from top to bottom, apoLp-I and apoLp-II). Large open arrow: albumin. Smaller open arrow: traces of albumin in the upper portion of the gradient.

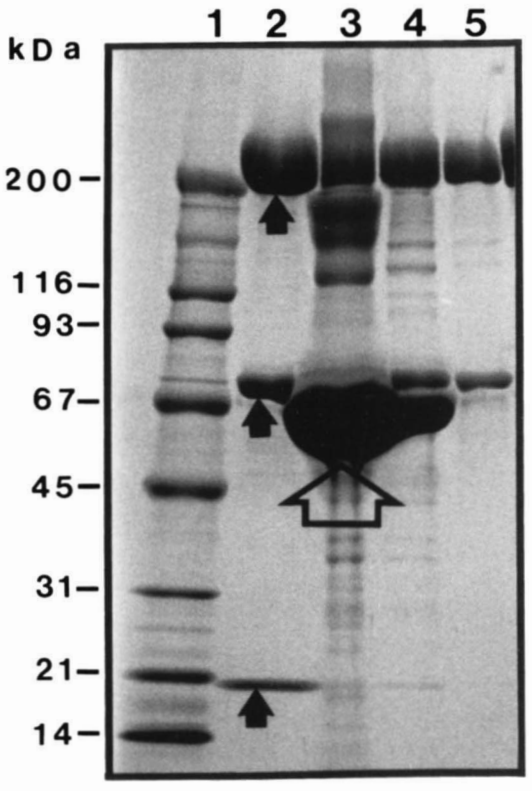

Fig. 3. Purification of Lp-5 after treatment of HDLp-A with lipase. HDLp-A (6 mg) was incubated with 200 mg of fatty acid-free albumin in the presence of 1 mg TAG-lipase. Following incubation (3 h, 34°C) the medium was subjected to density gradient ultracentrifugation as described under Experimental Procedures. Fractions containing lipophorin (as determined by SDS-PAGE) were pooled and subjected to affinity chromatography as described under Experimental Procedures. (1) Molecular weight markers; (2) HDLp-A prior to incubation with albumin and lipase. Solid arrows show the three apolipoproteins of lipophorin (as defined in legend to Fig. 2); (3) Total medium after incubation of HDLp-A with albumin and TAG-lipase. Open arrow shows albumin; (4) Lp-5 after density gradient ultracentrifugation; (5) Lp-5 after thiophilic affinity chromatography.

lipase. Native LDLp particles were round and quite heterogeneous in size with diameters ranging from 20 to 32 nm (average diameter ~25 nm; **Fig. 4A**). HDLp-A, on the other hand, was more homogeneous in size with diameters ranging from 15 to 17 nm (average diameter ~16 nm; Fig. 4B). When LDLp was treated repeatedly with TAG-lipase to generate Lp-3, the diameters of the lipoproteins decreased to values ranging from 12 to 15 nm (average diameter ~14 nm). Although Lp-3 appears to be less spherical than either LDLp or HDLp-A, Fig. 4C clearly demonstrates the structural integrity of Lp-3. The higher values for lipophorin diameters obtained by electron microscopical analysis, compared to those obtained by gel permeation chromatography (Table 1), are explained by the tendency of lipoproteins to flatten during sample preparation for negative staining (39).

Percentages of radiolabeled DAG and total DAG during stepwise incubations of LDLp with TAG-lipase

Table 2 shows the relationship between radiolabeled DAG and total DAG in LDLp before and after treatment with TAG-lipase. The shift in the ratio of % radiolabeled DAG in the particle to total lipophorin-bound DAG shows that radiolabeled DAG was depleted from the particle more rapidly than the total DAG fraction.

DISCUSSION

Insect lipophorin is a lipoprotein that can undergo many changes in its composition under different physiological or developmental conditions. Variations in lipid load result in lipophorin particles of which the density ranges from 1.238 g/ml (VHDLp from the egg, 40) to 1.030 g/ml (the flight-specific LDLp, 13). The results presented above show that lipids can also be selectively un-

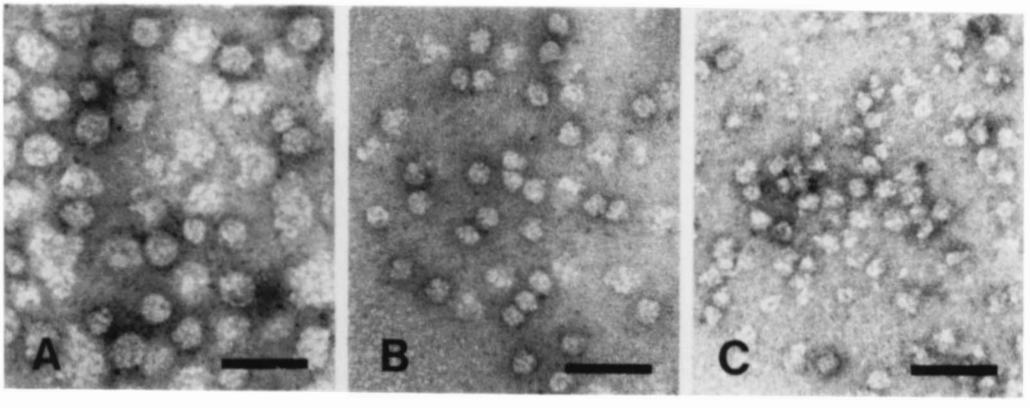

Fig. 4. Electron micrographs of negatively stained native and lipase-treated lipophorins. LDLp was treated repeatedly with TAG-lipase to generate Lp-3 (as described under Experimental Procedures). The isolated particles were negatively stained and analyzed by electron microscopy. A: LDLp, B: HDLp-A, C: Lp-3; bar scale = 500 Å.

TABLE 2. Percentage of radiolabeled DAG and total DAG in LDLp during step-wise incubation of LDLp with TAG-lipase

Particle	Radiolabeled DAG	Total DAG	Apparent d	Radius
	%/particle		*g/ml*	*Å*
LDLp*	98	47	1.052	84
Lp*-7	39	34	1.102	78
Lp*-8	5	27	1.136	64
Lp*-9	≤1	17	1.157	58

Lipophorin that contained tritium-labeled DAG (LDLp*) (9.4 mg) was incubated with 2 μg of TAG-lipase and 200 mg of albumin for 1 h. Following incubation, lipophorin (Lp*-7, 7.6 mg) was isolated from the mixture and incubated for 1 h with 2 μg of lipase in the presence of 200 mg albumin. After incubation, Lp*-8 (5.9 mg) was retrieved from the medium and incubated with 10 μg of lipase and albumin (200 mg for 1 h). The Lp*-9 obtained from the final incubation, together with LDLp*, Lp*-7, and Lp*-8 were analyzed for the amount of radiolabel in the DAG fraction. The sizes and densities of the particles were determined. Values represent the mean of three determinations; standard deviations were ≤5% for radioactivity determinations and between 1% and 7% for radius determinations. Density determinations varied less than 0.004 g/ml.

loaded from lipophorin in vitro using lipolytic enzymes. Unlike the situation in vivo, an array of lipid-depleted lipophorin particles can be obtained in vitro by varying the lipophorin/lipase ratio. The resulting particles were used not only to examine the process by which lipids are removed from lipophorin, but also to analyze the structure of lipophorin at various stages of lipid unloading.

Localization of PL

It has been suggested that PL are located at the surface of lipophorin (18), as is also the case in human lipoproteins (21–23). This is supported by the accessibility of lipophorin PL to PLA_2 as we have shown in this report. Even with its dramatically increased content of DAG and apoLp-III, the PL of LDLp remains completely accessible to PLA_2 (Table 1), suggesting that PL resides in the outermost layer of the particle. Therefore, lipid loading of HDLp-A to LDLp apparently involves a reorganization of the surface layer so that PL remains at the surface.

Localization of DAG

Calculations based upon the volume and composition of *M. sexta* HDLp led to the conclusion that lipophorin DAG was partitioned between the hydrophobic core and the hydrophilic surface of the particle (17), much as the partitioning of free cholesterol in human lipoproteins (20–22). However, Katagiri, Sato, and Tanaka (19) suggested that the DAG fraction of the locust lipophorin is sandwiched between the hydrophobic core of hydrocarbon and the surface layer of PL and apoproteins. The results reported here, demonstrating rapid hydrolysis of lipophorin DAG by lipase, show that some of the DAG is readily accessible to the enzyme and is, therefore, localized at the particle-water interface. In the present study, however, large amounts of DAG could be removed from both LDLp and HDLp-A, resulting in very high density particles. The limited depletion of DAG from the surface may have altered the equilibrium between DAG in the core and DAG at the surface. In order to restore the equilibrium, some of the core DAG may have moved to the surface, thus becoming susceptible to lipase.

If this model of lipid unloading, in which DAG migrates from a core or an intermediate layer into the outer surface layer to replace DAG eliminated by the action of lipase, is true, there must be some distribution of DAG in two or more pools that do not equilibrate rapidly. Our experiments show that labeled DAG, loaded onto lipophorin in vivo (to produce LDLp that contains label only in DAG), was eliminated by TAG-lipase more readily than unlabeled DAG that existed in the parent HDLp-A (see Table 2). This is best explained by assuming that most of the labeled DAG was localized at the surface layer and was not uniformly equilibrated with DAG in the core of the particle.

Function of PL and DAG in the stabilization of lipophorin

Since PL exists in a stable orientation at the air-water interface, one would expect PL to play a major role in stabilizing lipophorin in the aqueous phase of the hemolymph. Although Katagiri (18) reported that locust lipophorin remains stable following treatment with phospholipase, he did not isolate the PL-depleted lipophorin, nor were the lipid products of the enzyme-catalyzed reaction removed. Since it has been shown that lysophospholipids are detergents with excellent capacity to stabilize apolipophorins in solution (33), Katagiri's study did not prove the stability of PL-depleted lipophorins. As reported here, the products resulting from treatment of lipophorins with PLA_2 can be completely eliminated by albumin, and the resulting particles, free of all PL except SP, are stable in aqueous solution. These results indicate that PL are not necessary for the stability of lipophorin. It could be argued that SP, which constitutes about 5% of the lipophorin PL, is sufficient to stabilize the glycerophospholipid-depleted particle, but we consider this an unlikely explanation because this lipid occupies <1% of the total surface area of the particle. When lipophorin was first depleted of DAG and then treated with PLA_2, the particle became unstable. Apparently, in the absence of DAG, PL is the major stabilizing element in the outer shell of lipophorin.

Electron microscopical analysis confirmed the structural integrity of the highly DAG-depleted Lp-3. Nevertheless, Lp-3 had the tendency to aggregate at higher protein concentrations, presumably because of hydrophobic effects that could result from exposure of the hydrophobic inner core of lipids to the aqueous environment, or because of a rearrangement of the apolipoprotein chains to

expose hydrophobic residues, or both. Nascent lipophorin, secreted by the fat body (41), is a VHDLp similar in size to Lp-3, rich in PL, and deficient in acylglycerols. The PL content of VHDLp seems to be sufficient to stabilize the particle until it can take on a complement of DAG. During lipid transport, the PL complement of lipophorin appears to remain rather constant while that of DAG varies widely (13). The constant level of PL in lipophorin may ensure the stability of the particle in the aqueous phase when DAG content is extremely low, as, for example in nascent lipophorin and in egg VHDLp (40). When ample amounts of DAG are present in the outer shell, however, the particle may be stabilized by these molecules in virtual absence of PL.

Localization and function of apoLp-III in lipophorin

At the air–water interface, PL monolayers have a higher phase transition point than DAG monolayers (42). Based upon this and other structural differences between the two lipids, DAG may be less stable than PL at the lipophorin–water interface. Therefore, the presence of a large amount of DAG at the surface of LDLp (up to 4 times that in HDLp-A, Table 1) would destabilize the particle in aqueous solution. However, LDLp remains stable, and this is presumably the result of the association of several molecules of apoLp-III with the particle (11). The actual ligand for apoLp-III (if a specific one exists) at the lipophorin–water interface has not been established. Kawooya et al. (43) studied the interaction of apoLp-III with artificial lipid surfaces. In that study, apoLp-III was reported to bind to both PL- and DAG-coated polystyrene beads, as well as to beads coated with a mixture of both lipids. We have shown here that although treatment of HDLp-A with PLA_2 results in dramatic depletion of PL from lipophorin, it does not lead to the elimination of apoLp-III. However, removal of substantial amounts of DAG by TAG-lipase does result in dissociation of apoLp-III from lipophorin. This could be interpreted as evidence for the association of apoLp-III with lipophorin-bound DAG, and, therefore, supports the proposed role of apoLp-III in stabilizing DAG-enriched forms of lipophorin.

Conclusions

Experiments reported here show that the basic matrix of lipophorin is resilient and can maintain its integrity even when grossly depleted of PL or DAG, but not when both lipids are eliminated from the particle. This indicates that both PL and DAG play a role in lipophorin stability. The results further show that PL, apoLp-III, and part of the DAG are located at the surface of the particle as has been suggested by others. Most importantly, we have shown for the first time that at least two pools of DAG exist, that do not equilibrate rapidly. The tolerance for large changes in lipid volume is a prerequisite for a particle whose primary physiological function is to shuttle lipids between tissues. Although the results reported here show that lipophorin can be largely depleted of DAG or PL, they do not prove that the unloaded particles are able to reload these lipids. This aspect of the shuttle function of lipophorin is approached in the accompanying report (44).

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