Effect of phospholipase C and apolipophorin III on the structure and stability of lipophorin subspecies

T. K. Amareshwar Singh, Hu Liu, Roger Bradley, Douglas G. Scraba, and Robert O. Ryan[1]

Department of Biochemistry and Lipid and Lipoprotein Research Group, University of Alberta, Edmonton, Alberta, Canada T6G 2S2

Abstract Four distinct subspecies of the insect hemolymph lipoprotein, lipophorin, that range in diacylglycerol (DAG) content from approximately 100 to 1000 molecules per particle, were treated with phospholipase C. Lipid analysis demonstrated that both phosphatidylcholine and phosphatidylethanolamine were hydrolyzed to DAG. Phospholipase C was used to remove 74–82% of the phospholipid of different lipophorins and these were analyzed for aggregation. Low density lipophorin (LDLp), the largest subspecies, with a diameter of ~23 nm, developed turbidity (monitored by sample absorbance at 340 nm) suggesting the formation of lipoprotein aggregates. High density lipophorin-adult (HDLp-A) and high density lipophorin-wanderer 1 (HDLp-W1) also displayed an increase in A_{340} when incubated with phospholipase C, although the maximal increase observed was considerbly less than that for LDLp on a per particle basis. Phospholipase C caused only a minimal increase in A_{340} in a fourth subspecies, high density lipophorin-wanderer 2 (HDLp-W2), which contains an even lower amount of DAG. Electron microscopy was used to evaluate changes in particle morphology as a result of phospholipid depletion. HDLp-W2 and HDLp-W1 showed signs of progressive aggregation and particle fusion. A similar aggregation/fusion was seen in the case of high density lipophorin adult (HDLp-A) while LDLp samples contained multiple aggregation/fusion foci and resultant very large particles. In the presence of exogenous apolipophorin III (apoLp-III), phospholipase C-induced lipophorin aggregation/fusion was prevented. Electron microscopy of LDLp and HDLp-A samples revealed that apoLp-III-stabilized, phospholipase C-treated particles had a morphology similar to that of control particles. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of HDLp-W1, HDLp-A, and LDLp after incubation with phospholipase C and apoLp-III demonstrated the association of apoLp-III with these lipoproteins. Scanning densitometry of the stained gels showed that phospholipase C-treated, apoLp-III-stabilized lipophorin samples acquired 3–5 apoLp-III molecules/particle as a result of phospholipase C-catalyzed phospholipid conversion to DAG. Thus, these experiments establish a correlation between the generation of DAG and the binding of apoLp-III to lipophorin particles. Furthermore, they provide direct evidence that association of apoLp-III with DAG-enriched lipophorins functions to stabilize particle structure. —**Singh, T. K. A., H. Liu, R. Bradley, D. G. Scraba, and R. O. Ryan.** Effect of phospholipase C and apolipophorin III on the structure and stability of lipophorin subspecies. *J. Lipid Res.* 1994. **35:** 1561–1569.

Supplementary key words diacylglycerol • phosphatidylcholine • electron microscopy • phosphatidylethanolamine • insect • *Manduca sexta*

Due to their water insolubility, lipids are transported in plasma by packaging them into lipid-protein assemblies. All organisms that possess a circulatory system use lipoproteins to transport lipids from their sites of origin to sites of utilization or storage. Common structural features shared by lipoproteins include a core of apolar lipid surrounded by a monolayer of amphiphilic apoproteins, phospholipid, and unesterified cholesterol. In general, alterations in either core lipid content or surface components can have a significant effect on the structure and stability of lipoproteins.

Experimental studies of facilitated transfer of diacylglycerol (DAG) from insect lipophorin donor particles to human low density lipoprotein (LDL) showed that LDL is an excellent acceptor of lipophorin-derived DAG in vitro (1). When the amount of DAG transferred exceeds the capacity of LDL to accept it, LDL aggregates form (2). Aggregation can be prevented, however, by co-incubation with apolipophorin III (apoLp-III), a water-soluble, 18 kDa, surface-binding apolipoprotein (3–5). Analysis of apolipoprotein-stabilized, DAG-enriched LDL particles showed that binding of amphipathic apolipoproteins compensated for the increase in hydrophobic core lipid molecules (2). A similar naturally occurring physiological phenomenon is the adipokinetic hormone-induced, flight-related, lipid loading of lipophorin in insects such as *Manduca sexta* and *Locusta migratoria.* During flight, lipophorin DAG content increases by several fold and high density lipophorin-adult (HDLp-A) is converted into low density lipophorin (LDLp), which gains 9–14 additional apoLp-III molecules (see refs. 6, 7 for reviews). Accompanying these compositional changes is an increase in particle diameter from 16 nm to ~23 nm (8) and a

Abbreviations: DAG, diacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; apoLp-III, apolipophorin III; HDLp-W2, high density lipophorin-wanderer 2; HDLp-W1, high density lipophorin-wanderer 1; HDLp-A, high density lipophorin adult; LDLp, low density lipophorin.

[1]To whom correspondence should be addressed.

decrease in density from 1.08 to 1.03 g/ml (9). On the basis of these data and ^{31}P-NMR studies, we proposed a model for the interaction between apoLp-III and a DAG-enriched lipophorin (10).

Enzymatic depletion of the phospholipid moiety of lipoproteins has also been used to alter their structures. Although deletion of one fatty acyl chain by phospholipase A_2 hydrolysis induces only modest changes when compared with native lipoproteins (11–13), profound changes in human LDL have been observed after removal of polar head groups of phospholipids by phospholipase C or sphingomyelinase (14, 15). When LDL is subjected to enzymatic treatment by either phospholipase C or sphingomyelinase, the particles aggregate. Binding of either human apoA-I or insect apoLp-III prevents LDL aggregation induced by phospholipase C (16). In the present study we chose four insect lipophorin species that have different morphologies (17) and contain different amounts of DAG as their major core lipid (18), and we studied the ability of these lipoproteins to tolerate enzymatic creation of DAG at the expense of phospholipid. A correlation between DAG generation/phospholipid depletion and apoLp-III binding was found; this supports established hypotheses regarding the mode of apoLp-III association with the surface of lipophorin.

EXPERIMENTAL PROCEDURES

Materials

Phospholipase C from *Bacillus cereus* (Grade I; <0.05% sphingomyelinase activity) was purchased from Boehringer Mannheim. Larval and adult *Manduca sexta* were obtained from a continuing laboratory colony reared on a wheat germ-based diet according to Prasad et al. (19). Lipophorins were isolated from freshly collected hemolymph of larval or adult *M. sexta* by density gradient ultracentrifugation (20). High density lipophorin wanderer 2 (HDLp-W2) and high density lipophorin wanderer 1 (HDLp-W1) were isolated from prepupal larvae as described by Prasad et al. (19). HDLp-A and LDLp were isolated from 1-day-old adult moths according to Ryan et al. (9). ApoLp-III was purified from adult hemolymph as described by Wells et al. (21).

Phospholipase C assay

Routine assays were conducted at 37°C in microtiter plates containing 150 μg lipophorin phospholipid in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 2 mM Ca^{2+} in the presence or absence of exogenous apoLp-III. Reactions were initiated by the addition of phospholipase C and stopped by the addition of an aliquot of 50 mM EDTA. The optimal wavelength for turbidity detection was determined from difference spectra comparing incubations of each lipophorin in the presence and absence of phospholipase C. The spectra revealed a similar trend for each subspecies, with the maximal absorbance difference occurring between 340 nm and 450 nm. Subsequently, absorbances were measured at 340 nm on an SLT Lab instruments microtiter plate reader.

Analytical procedures

Protein content was determined with the bicinchoninic acid assay (Pierce Chemical Co.), using bovine serum albumin as standard. Lipid contents were analyzed by an enzymatic kit for glycerolipid (from Boehringer Mannheim) and by inorganic phosphorus analysis for phospholipids (22). In some cases lipids were extracted with chloroform–methanol 2:1 and separated by thin-layer chromatography using plates coated with silica gel G. Samples were separated in a solvent system containing chloroform–methanol–acetic acid–water 50:38:2:10 and visualized by exposure to iodine vapors. SDS-PAGE was performed using 4–20% acrylamide gradient slab gels. After staining with Coomassie Brilliant Blue, gels were scanned on a Cannag TLC Scanner II. Electron microscopy was performed as previously described (23).

RESULTS

Phospholipase C treatment of lipophorin subspecies

The phospholipid moiety of lipophorin forms a monolayer on the particle surface (24). Attack by phospholipase C results in the release of polar head groups and the in situ generation of DAG, which is the major neutral lipid component of lipophorins. As a multitude of lipophorin subspecies exist which possess dramatically different lipid compositions (18, 25), we set out to determine the effect of phospholipase C treatment on the extent of phospholipolysis, changes in particle morphology, and sample turbidity. To determine which phospholipids were hydrolyzed, control and phospholipase C-treated lipophorins were extracted and analyzed by thin-layer chromatography. Compared to controls, incubation of lipophorins in the presence of phospholipase C resulted in a significant reduction in phosphatidylcholine (PC) and phosphatidylethanolamine (PE) content, while sphingomyelin was unaffected. In all cases depletion of lipophorin PC and PE content was accompanied, as expected, by an increase in DAG. As sphingomyelin represents a relatively minor phospholipid component of *M. sexta* lipophorin (10), these data indicate that a significant portion of lipophorin phospholipid is susceptible to phospholipase C hydrolysis.

Incubation of each of four distinct lipophorin subspecies (150 μg phospholipid) for 90 min with 3 units phospholipase C resulted in variable amounts of phospholipid hydrolysis and sample turbidity development (**Table 1**). Compared to other subspecies, HDLp-W2 appeared to be resistant to phospholipase C-catalyzed hydrolysis (27%

TABLE 1. Effect of phospholipase C treatment on lipophorin subspecies

Subspecies	Original Composition (%)[a] DAG[a]	Original Composition (%)[a] Phospholipid[a]	Phospholipase C-treated % Phospholipid Hydrolyzed[b]	Phospholipase C-treated ΔA_{340}
HDLp-W2	12.5	19	82.0 ± 2.2	0.01
HDLp-W1	20	23	74.4 ± 3.7	0.08
HDLp-A	25	14	76.8 ± 3.4	0.04
LDLp	47	7	84.2 ± 1.5	0.53

[a]HDLp-W2 and HDLP-W1 composition (weight percent) is from Prasad et al. (19) while that for HDLp-A and LDLp is from Ryan et al. (9).
[b]HDLp-WI, HDLp-A, and LDLp incubations (90 min, 37°C) contained 3 units phospholipase C and 150 μg lipophorin phospholipid. Incubations with HDLp-W2 (5 h, 37°C) contained 5 units enzyme and 150 μg phospholipid. Values represent the mean ± standard deviation (n = 3).

hydrolysis under these conditions). Increasing the amount of phospholipase C to 5 units and extending the incubation time to 5 h, however, resulted in conversion of 82% of HDLp-W2 phospholipid to DAG (Table 1). Even with this extent of phospholipolysis, however, virtually no increase in sample turbidity was observed. The effect of phospholipid conversion to DAG on the morphology of HDLp-W2 was examined by electron microscopy (**Fig. 1**). Low magnification micrographs of control HDLp-W2 (Fig. 1A) revealed a homogeneous particle population. At higher magnification (Fig. 1B) these particles display a hamburger-like morphology, being oblate spheres with a distinctive central cleft (as reported earlier; 17). In the case of phospholipase C-treated HDLp-W2, conversion of 82% of its phospholipid complement to DAG revealed

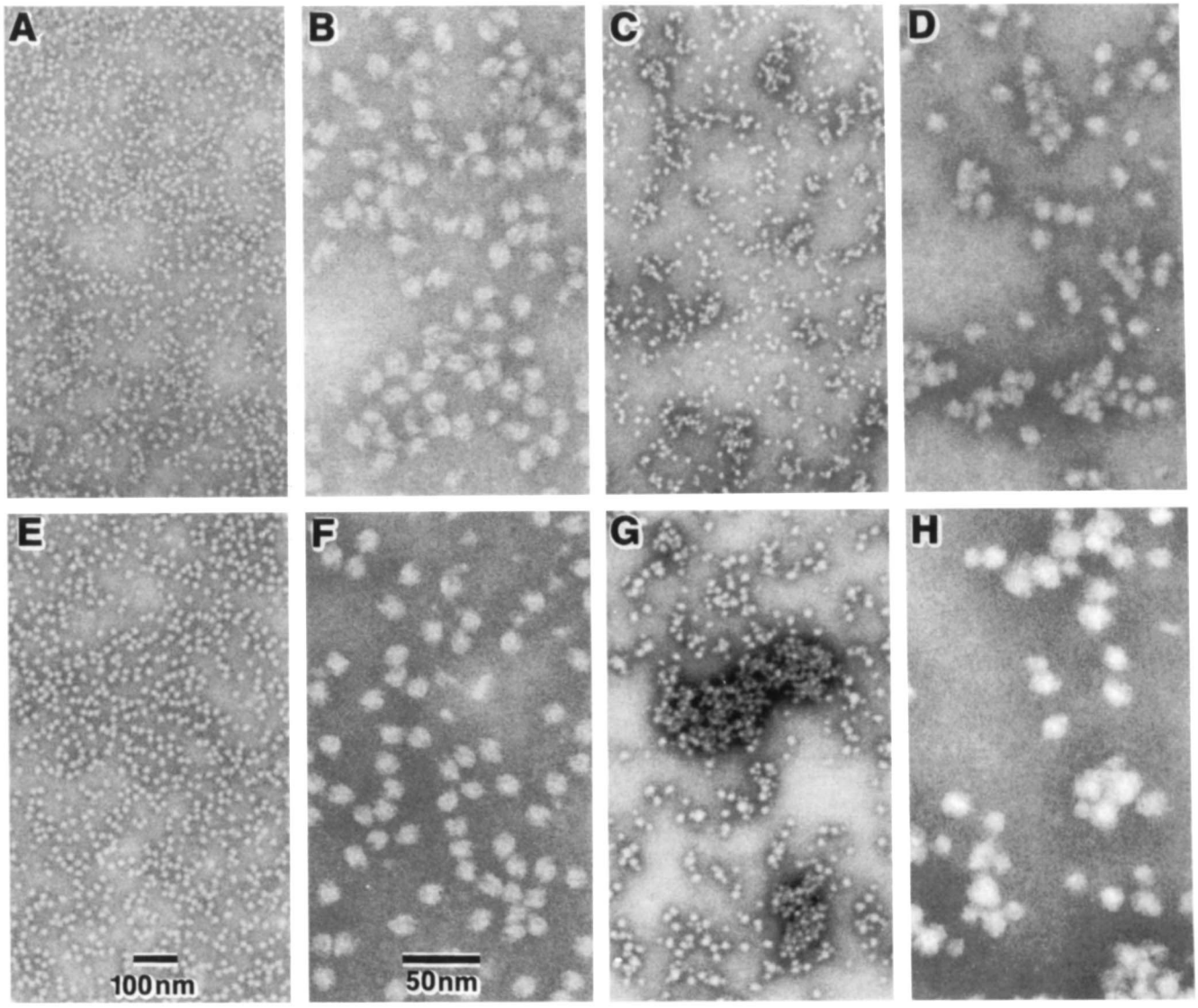


Fig. 1. Electron microscopy of phospholipase C-treated lipophorins. Lipophorin samples (150 μg phospholipid) were incubated in the absence and presence of phospholipase C as described in Experimental Procedures. The samples were negatively stained with 2% sodium phosphotungstate and photographed in a Philips EM420 operated at 100 kV. Panels A and B) HDLp-W2 control at low and high magnification; panels C and D) HDLp-W2 plus phospholipase C (5 units, 5 h incubation); panels E and F) HDLp-W1; panels G and H) HDLp-W1 plus phospholipase C (3 units, 1.5 h).

some clumping of particles (Fig. 1C); this limited aggregation, however, was not reflected in the A_{340}. At higher magnification (Fig. 1D), the lipolyzed HDLp-W2 particles show clear signs of aggregation and unassociated particles display a more rounded appearance than controls.

Phospholipase C treatment of HDLp-W1 induced 74% phospholipid hydrolysis and a small, but reproducible, increase in sample turbidity (Table 1). These data suggest that the phospholipid component of HDLp-W1 is more susceptible to phospholipase C hydrolysis than is that of HDLp-W2. Electron micrographs (Fig. 1E, F) indicate that control HDLp-W1 are monodisperse, uniform size particles that possess morphological characteristics similar to the HDLp-W2 particles (17). Micrographs of phospholipase C-treated HDLp-W1 (Fig. 1G, H), however, revealed evidence of particle aggregation, fusion, and alteration of morphology, indicating fusion events had occurred.

In the case of HDLp-A, electron microscopy (**Fig. 2A**) revealed a sphere-like morphology with a particle diameter of 16 nm (17). Phospholipase C treatment of this subspecies induced hydrolysis of 77% of its phospholipid complement and an increase in A_{340} of 0.04 (Table 1). Micrographs of phospholipase C-treated HDLp-A (Fig. 2B, E) revealed a lesser extent of aggregation/fusion than displayed by HDLp-W1, although the extent of phospholypolysis was similar.

LDLp is the largest lipophorin subspecies and contains the highest ratio of DAG:phospholipid (7.5:1). This particle is known to be relatively unstable and to exhibit size heterogeneity (8). Electron micrographs of control LDLp are consistent with previous work (Fig. 2G, J) and revealed particles with an average diameter of 19 nm. Treatment of LDLp with phospholipase C resulted in conversion of 84% of its phospholipid complement into DAG and induced a large increase in A_{340}, suggesting that particle fusion occurred. This was confirmed by electron microscopy (Fig. 2H, K) which revealed the presence of multiple foci of fused complexes and creation of products with diameters >50 nm. This result is consistent with the increased absorbance of this sample compared to control LDLp. In addition, however, the sample revealed many particles that were of normal size indicating that, following this extent of phospholipolysis, many particles retained their native morphology.

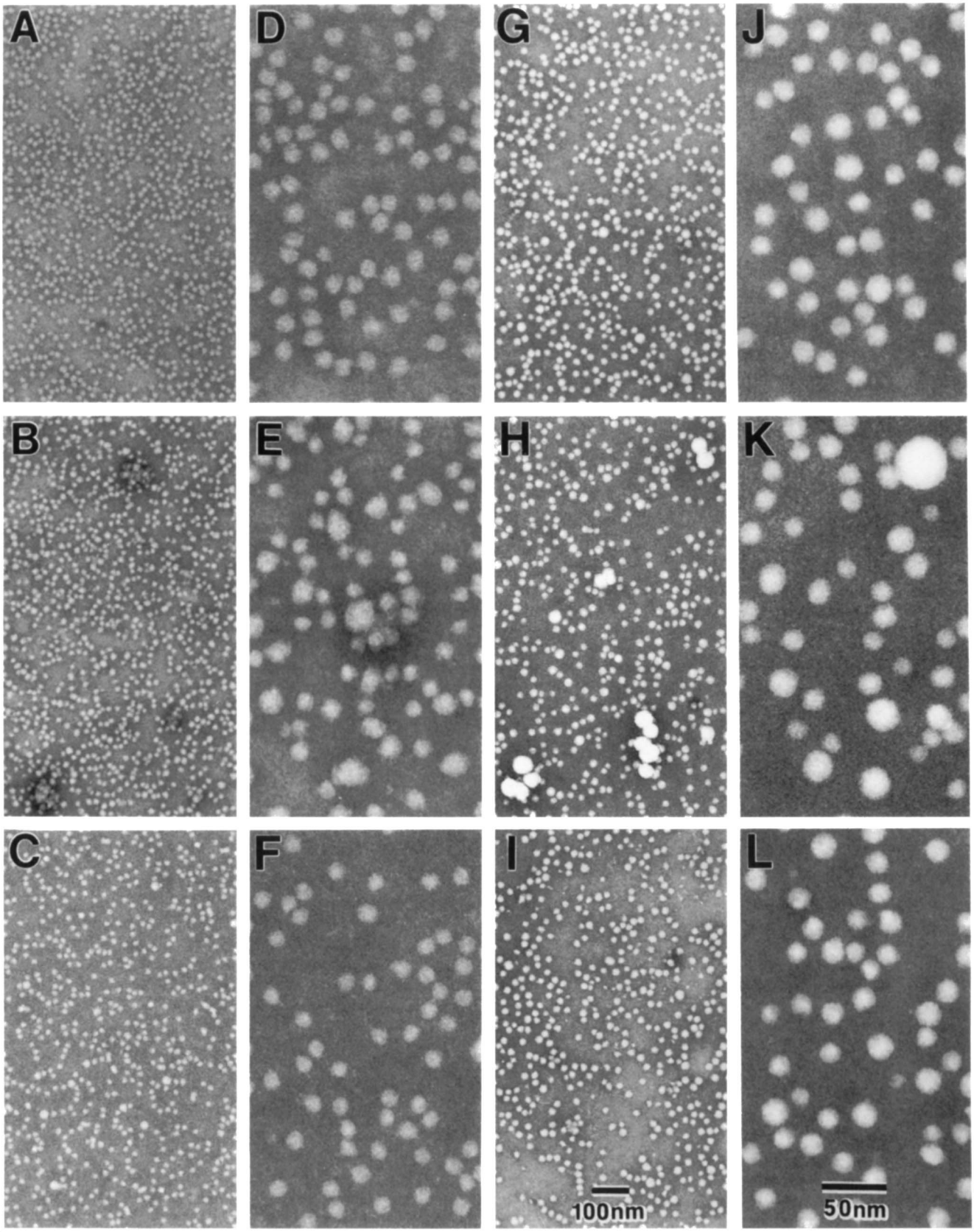
Prevention of phospholipase C-induced lipophorin aggregation by apoLp-III

During hormone-stimulated creation of LDLp from HDLp-A in vivo, the dramatic increase in particle DAG content is accompanied by binding of apoLp-III to the particle surface, where it is postulated to stabilize the DAG-enriched structure (6). As phospholipase C treatment of lipophorins results in an increase in DAG content and, in some cases, particle instability, we hypothesized that apoLp-III could prevent phospholipase C-induced lipophorin aggregation. When co-incubated with LDLp, apoLp-III prevented sample turbidity development in a concentration-dependent manner (**Fig. 3**) although the extent of phospholipolysis was unaffected (data not shown). To verify that apoLp-III maintained particle integrity in spite of phospholipid hydrolysis, electron microscopy was used. When apoLp-III was present in incubations of HDLp-A or LDLp and phospholipase C, particle integrity was maintained (Fig. 2, panels C, F, I, L) and the particles in the treated sample displayed a morphology similar to control particles. Addition of apoLp-III to phospholipase C-treated, turbid LDLp had no effect, indicating that apoLp-III cannot reverse the aggregation process. On the basis of these results we hypothesize that apoLp-III-mediated prevention of phospholipase C-induced lipophorin sample turbidity development occurs via formation of a stable binding interaction.

Association of apoLp-III with phospholipase C-treated lipophorins

Each lipophorin subspecies was incubated with apoLp-III in the absence and presence of phospholipase C, followed by density gradient ultracentrifugation to remove unbound apoLp-III. Under these conditions lipophorin samples float above lipid-free apoLp-III, which remains in the bottom fraction. Lipophorin samples reisolated in this manner were subjected to SDS-PAGE (**Fig. 4**). Compared to the control, phospholipase C did not induce binding of apoLp-III to HDLp-W2. ApoLp-III was found in association with HDLp-W1, however, following phospholipase C treatment. Furthermore, although apoLp-III is normally found in association with both HDLp-A and LDLp, phospholipase C treatment of these lipophorins induced additional apoLp-III binding. Using apoLp-II as

Fig. 2. Electron microscopy of phospholipase C-treated lipophorins. Lipophorin samples (150 μg phospholipid) were incubated in the absence and presence of phospholipase C as described in Experimental Procedures. The samples were stained and photographed as described in the legend to Fig. 1. Panels A and D) Control HDLp-A at low and high magnification; panels B and E) HDLp-A plus phospholipase C (3 units, 1.5 h); panels C and F) HDLp-A plus phospholipase C and apoLp-III (200 μg); panels G and J) control LDLp; panels H and K) LDLp plus phospholipase C (3 units, 1.5 h); panels I and L) LDLp plus phospholipase C and apoLp-III (200 μg).



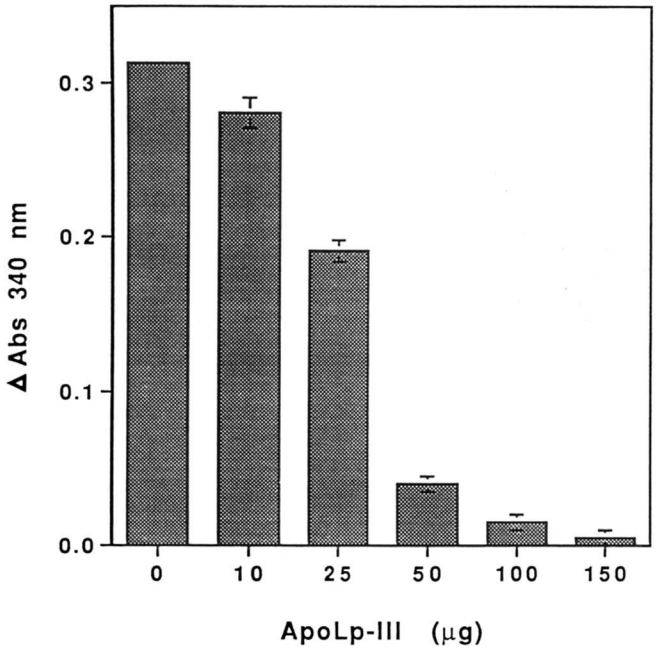


Fig. 3. Prevention of phospholipase C-induced lipophorin sample turbidity development by apoLp-III. LDLp (50 μg phospholipid) was incubated in the presence and absence of phospholipase C (1 unit) at 37°C in the presence of indicated amounts of apoLp-III. A_{340} values reported were obtained after 4 h incubation. For each apoLp-III concentration the A_{340} of the control incubation was subtracted from the absorbance of the corresponding samples containing phospholipase C. Values reported are the average of three determinations ± standard deviation (n = 3).

internal standard, the amount of apoLp-III in each lane of the gel was estimated by scanning densitometry. Based on the knowledge that native HDLp-A contains 2 apoLp-III and LDLp contains, on average, 16 apoLp-III molecules per particle (26), we estimate that phospholipase C treatment induces association of 3–5 more apoLp-III molecules per HDLp-W1, HDLp-A or LDLp particle.

DISCUSSION

Lipophorin subspecies naturally contain different amounts of DAG as their major core lipid while maintaining the same integral apolipoprotein components, apoLp-I and apoLp-II (M_r = 240,000 and 80,000, respectively; 6). It has been proposed that apoLp-I and apoLp-II, together with phospholipids, comprise a matrix structure that is common to all forms of lipophorin. At different life stages of the insect this structural framework allows neutral lipids to be loaded onto or removed from lipophorin to meet different metabolic lipid transport requirements (6, 17). Phospholipase C digestion of lipophorin phospholipids results in loss of the polar head groups of both PC and PE. Accumulation of the product DAG, owing to its smaller and far less polar head group, destabilizes the surface monolayer of lipoproteins. Indeed, it has been shown that incorporation of small amounts of DAG into bilayer membranes promotes a lamellar to reverse hexagonal II phase transition (27, 28). Likewise, phospholipase C hydrolysis of the PC component of human LDL induces instability and particle aggregation (14, 16).

The observation that lipophorin exists as several distinct subspecies suggests that its apolipoprotein matrix has the capacity to undergo structural and conformational changes that accommodate changes in lipid composition. Previous work has revealed that HDLp-W2 possesses a central cleft or cavity, suggesting that the particle core is not fully occupied by neutral lipid (17). As native HDLp-W2 contains relatively little DAG (100 DAG and 134 phospholipid molecules per particle; 19), it is conceivable that DAG molecules created by phospholipid hydrolysis could partition into available space in the particle core. In other words, since HDLp-W2 possesses an apparent excess of surface components as well as spare core capacity, conversion of phospholipid to DAG may be tolerated because changes in conformation of the apolipoprotein matrix permit maintenance of particle structure through sequestration of DAG. Although the results from electron microscopy of phospholipase C-treated HDLp-W2 indicate their cavities have been filled, there is also some particle clumping.

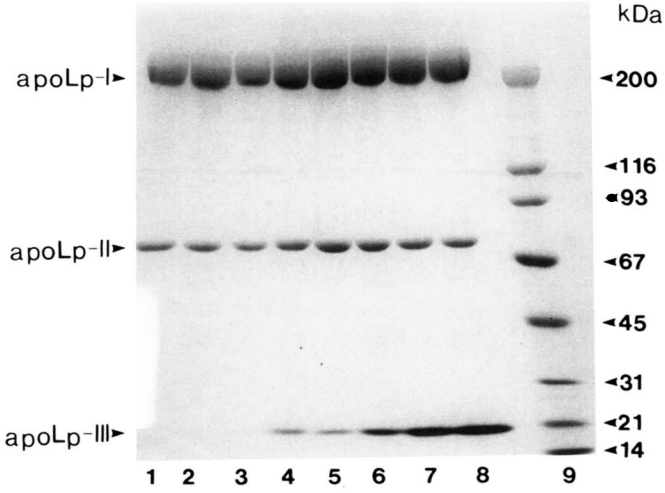


Fig. 4. The effect of phospholipase C on apoLp-III binding to lipophorins. Four hundred μg protein of each of the indicated lipophorin subspecies was incubated with 400 μg apoLp-III in the absence and presence of 8 units of phospholipase C at 22°C for 12 h. The reaction was stopped by adding 100 μl 50 mM EDTA. The samples were adjusted to a density of 1.21 g/ml with KBr and subjected to density gradient ultracentrifugation. After fractionation the isolated lipophorins were electrophoresed on a 4–20% acrylamide gradient, SDS slab gel. Lanes 1, 3, 5, and 7 correspond to HDLp-W2, HDLp-W1, HDLp-A, and LDLp, respectively, incubated without phospholipase C; lanes 2, 4, 6, and 8 are HDLp-W2, HDLp-W1, HDLp-A, and LDLp, respectively, after incubation with phospholipase C. Lane 9 contains molecular weight standards.

HDLp-W1 is metabolically derived from HDLp-W2 through acquisition of more DAG (approximately 90 mol/mol lipophorin) and phospholipid (60 mol/mol lipophorin) causing changes in particle morphology toward a more uniformly spherical structure (17). Thus we postulate that, upon conversion of HDLp-W1-associated phospholipid to DAG, the capacity of the particle core is easily surpassed and DAG accumulates in the surface monolayer. It is also plausible that the loss of phospholipid head groups leads to a depletion of those surface components to an amount below that required for the maintenance of particle integrity. These processes, either independently or in concert, are responsible for the particle aggregation observed upon phospholipase C treatment. A similar interpretation can apply to HDLp-A, which, compared to HDLp-W1, displayed a lower propensity to aggregate after phospholipid conversion to DAG in spite of the fact that it contains more DAG per particle (10). The apparent resistance to aggregation/fusion of HDLp-A compared to HDLp-W1 may be attributable to a lower content of phospholipid per particle (134 verus 195) as well as the presence of two apoLp-III molecules on the surface (3).

During flight, DAG enrichment of HDLp-A creates LDLp particles. In this process lipophorin increases in diameter from 16 nm to ~23 nm and decreases in density from 1.08 to 1.03 g/ml (8, 9). Compositional analysis shows LDLp contains approximately 1,000 DAG molecules per particle. Compared to the HDLp forms, phospholipase C treatment of the larger, DAG-enriched LDLp induces a large increase in sample turbidity. This result was seen when phospholipase C assays were normalized on the basis of lipophorin particle protein, phospholipid, or DAG content and suggest that turbidity development in LDLp is a function of its larger size and neutral lipid content. Tentative conclusions drawn from increased LDLp sample turbidity are supported by results of electron microscopy. Unlike control LDLp, phospholipase C-treated LDLp can form fused aggregates that are likely responsible for the increased light scattering of the sample.

During formation of LDLp in vivo, the dramatic increase in DAG is not accompanied by an increased phospholipid content per particle (which remains constant at ~140 molecules). It has been postulated that the massive uptake of DAG during conversion of HDLp-A to LDLp results in partitioning of some DAG to the particle surface. Furthermore, it is proposed that LDLp stability is maintained by association of apoLp-III with the particle surface (10, 25). The tertiary structure of lipid-free *L. migratoria* apoLp-III has been determined by X-ray crystallography (29). In its lipid-free state, apoLp-III exists as a five-helix bundle in which the hydrophobic faces of its amphipathic α-helices point inward and their hydrophilic faces are exposed to the aqueous medium. As a surface component of lipoprotein particles, it has been proposed that the helix bundle opens to expose its hydrophobic surfaces which are responsible for interaction with the lipid surface created by DAG uptake (29, 30). In vivo, when LDLp-associated DAG is lipolyzed at the flight muscle and removed from the particle, the size of LDLp decreases and apoLp-III dissociates. This process converts LDLp back into an HDLp which is free to begin another round of DAG loading at the fat body (6). Emerging from these data is the concept that apoLp-III association with lipophorin is closely linked to DAG accumulation in the particle surface. Thus it may be anticipated that specific production or enrichment of DAG would induce apoLp-III binding and retention of particle stability. This concept has been supported by experiments using *M. sexta* lipid transfer particle to facilitate DAG enrichment of human LDL in vitro. In these studies facilitated vectorial DAG transfer occurred from HDLp to human LDL and it was found that, at a 1:1 lipophorin: LDL protein ratio, LDL particles possess the capacity to accommodate available DAG molecules without loss of structural integrity (1). Beyond a threshold of DAG enrichment, however, LDL particles aggregate into large complexes, as was shown by electron microscopy (2). Interestingly, lipid transfer particle-mediated, DAG transfer-induced LDL aggregation did not occur when apoLp-III was included in the incubation mixture. Instead, larger DAG and apoLp-III-enriched VLDL-like particles were isolated after gel filtration (2).

A similar lipoprotein aggregation phenomenon occurs when isolated human LDL is treated with phospholipase C (14) or sphingomyelinase (15). Also in this case, phospholipase C-induced LDL aggregation was prevented when amphipathic apolipoproteins, such as human apolipoprotein A-I or *M. sexta* apoLp-III, were included in incubations (16). Thus, it appears that distinct processes of lipoprotein DAG enrichment, including adipokinetic hormone stimulated LDLp formation in vivo, LTP-mediated vectorial transfer of DAG from lipophorin to human LDL, and phospholipase C-induced conversion of phosphatidylcholine to DAG on the surface of human LDL, are related insofar as each renders the substrate lipoprotein particle surface available for apoLp-III binding. We therefore consider that phospholipase C treatment of lipophorins may provide an experimental system to study factors that affect lipophorin-apoLp-III interactions. Indeed, phospholipase C-induced aggregation of HDLp-A and LDLp was prevented when incubations included apoLp-III. This prevention of particle aggregation/fusion by apoLp-III was not due to inhibition of phospholipase C activity but rather through enzyme reaction induced apoLp-III association with the lipophorin surface. Evidence was also obtained that phospholipase C treatment of HDLp-W1, HDLp-A, and LDLp induced association of 3–5 molecules of apoLp-III.

It is of interest to compare the results of the present

study to those reported by Kawooya et al. (13) using triacylglycerol lipase to specifically decrease the DAG content of HDLp-A and LDLp. These workers showed that DAG depletion by triacylglycerol lipase resulted in a decreased particle size and increased density. Furthermore, apoLp-III, originally associated with both HDLp-A and LDLp, dissociated from the particles upon DAG depletion. This latter result supports the concept that apoLp-III interacts with DAG on the surface of lipophorin particles and is entirely consistent with our observed phospholipase C-induced apoLp-III binding to lipophorins. By contrast, Hiraoka and Katagiri (31) reported that, although treatment of *L. migratoria* LDLp with lipoprotein lipase resulted in depletion of 90% of its DAG and a large reduction in particle diameter, no apoLp-III dissociation occurred. These workers concluded that dissociation of apoLp-III from lipophorin is independent of DAG content. Further work will be required to reconcile this apparent discrepancy.

In other studies, phospholipase A_2 digestion of *M. sexta* HDLp-A and LDLp (13) or *Rhodnius prolixus* lipophorin (32) in the presence of fatty acid-free albumin (to remove free fatty acids and lysophosphatidylcholine generated by phospholipase A_2 activity) resulted in creation of stable phospholipid-depleted lipophorins. On the basis of these results the authors concluded that phospholipids are not necessary for maintenance of lipophorin stability. The retention of particle stability after phospholipase A_2-mediated depletion of phospholipid suggests that the apolipoprotein structural framework of lipophorin has sufficient inherent flexibility and/or reserve capacity to compensate for loss of phospholipid. It is conceivable that reorientation of apolipoproteins may permit them to cover a greater area of the particle surface. The inability to retain a stable structure following treatment with phospholipase C, however, may be attributable to the combined effect of phospholipid depletion coupled to accumulation of the product DAG, which remains associated with lipophorin, placing extra demands on residual surface components.

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