Effect of particle lipid content on the structure of insect lipophorins

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Abstract Four distinct subspecies of the major insect lipoprotein, lipophorin, that range in overall lipid content from 20 to 51% of the particle mass, were isolated from the hemolymph or oocytes of the tobacco hornworm, *Manduca sexta*. Examination of these subspecies by electron microscopy revealed distinctive morphologies. Adult high density lipophorin (HDLp-A) was found to be an approximately spherical particle with a diameter of 15 ± 1 nm, while HDLp-Wanderer 1 (W1), was more rectangular in shape and had a distinct cleft extending into the particle at one end. In the case of HDLp-Wanderer 2 (W2) the cleft was deeper and wider than that in HDLp-W1. In egg very high density lipophorin (VHDLp-E) the cleft was increased in size to the extent that the particle had an overall crescent-like conformation. Circular dichroism spectroscopy of the three lipophorin subspecies that contain only apolipophorin I and II revealed that only minor differences in the global protein secondary structure occur as the particle lipid content is decreased. The VHDLp-E apolipoproteins are an exception in that, while having the same α-helix content as HDLp-W1 and HDLp-W2, they contain less β–structure and correspondingly more random coil. Limited digestion of the apolipoprotein components of the lipophorin subspecies with trypsin revealed that as the lipid content of the particles decreases the susceptibility of the apolipoprotein to proteolytic degradation increases. Likewise, tryptophan fluorescence quenching experiments demonstrated that the relative exposure of lipophorin apolipoprotein tryptophan residues also increases as the particle lipid content decreases. Collectively, these experimental results provide evidence that naturally occurring lipophorin subspecies possess observable differences in morphology and structural organization, and imply that a flexible apolipoprotein structural framework encloses a central cavity into which lipid can be deposited and from which it can be removed. — **Ryan, R.O., C.M. Kay, K. Oikawa, H. Liu, R. Bradley, and D.G. Scraba.** Effect of particle lipid content on the structure of insect lipophorins. *J. Lipid Res.* 1992. **33:** 55–63.

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High density lipophorin (HDLp) is the major lipid transport vehicle in insect hemolymph. In the tobacco hornworm, *Manduca sexta,* lipophorin exists as distinct subspecies that contain different amounts of lipid while maintaining the same integral apolipoprotein components, apolipophorin I and apolipophorin II (M_r = 240,000 and 80,000, respectively) (1). It has been hypothesized that apoLp-I and apoLp-II, together with a small amount of phospholipid, comprise a stable basic matrix structure that is common to all forms of lipophorin (1). The basic matrix particle would therefore have the capacity to accept or donate variable amounts of lipid to produce the different lipophorin subspecies. The isolation and characterization of HDLp subspecies from different developmental stages of *M. sexta* has provided convincing evidence for the versatility of lipophorin with respect to lipid binding capacity (2, 3). For all the HDLp subspecies, protein constitutes between 48 and 70% of the particle mass; this being in the range of 500,000 to 800,000 daltons (4). In addition to apoLp-I and apoLp–II, adult high density lipophorin (HDLp-A) possesses two molecules of the low molecular weight apolipophorin III (M_r = 18,000) (5, 6). *M. sexta* oocytes (7) or hemolymph of larvae reared on a fat-free diet (8) contain a very high density lipophorin (VHDLp) that has 80% of its mass as protein in the form of apoLp-I and apoLp-II.

A distinguishing feature of lipophorins is the predominance of diacylglycerol as their major neutral lipid and a general lack of cholesteryl ester and triacylglcyerol, which are normal core components of mammalian lipoproteins. Although the presence of small amounts of long chain aliphatic hydrocarbon partially compensates for the lack of other nonpolar lipids, difficulties arise when attempts are made to fit

Abbreviations: HDLp-A, high density lipophorin-adult; HDLp-W1, high density lipophorin-Wanderer 1; HDLp-W2, high density lipophorin-Wanderer 2; VHDLp-E, very high density lipophorin-egg; apoLp, apolipophorin; PBS, phosphate–buffered saline; CD, circular dichroism.

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the known composition of these particles into structural models developed for mammalian lipoproteins. Compositional analyses of mammalian lipoproteins, together with physical measurements and morphological studies, fit well with a model in which hydrophobic lipids occupy the particle core and are surrounded by a monolayer of amphiphilic lipid and apolipoprotein, generating spherical structures (9). Similar analyses have been performed on lipophorin subspecies (4, 10); these are generally consistent with a partitioning of the bulk of the diacylglycerol as well as some portion of apoLp-II into the interior of the particle. A basic premise of these structural models of lipophorin, however, is that all of the subspecies are spherical. In this communication we present evidence that HDLp subspecies other than HDLp-A are not spherical, and that as the lipid content of the particles decreases the deviation from a sphere-like structure increases. At the same time the sensitivity of apolipophorins to proteolytic digestion and tryptophan fluorescence quenching increases, indicating increasing exposure of apolipoprotein as lipid is removed from the particle.

MATERIALS AND METHODS

Lipoprotein isolation

HDLp-A, HDLp-W1, HDLp-W2 were isolated from hemolymph and VHDLp-E was isolated from freshly dissected oocytes of the tobacco hornworm, *Manduca sexta*. The insects were obtained from a continuing laboratory colony reared on a wheat germ-based diet as described by Prasad et al. (2). Hemolymph samples were placed directly into phosphate-buffered saline (PBS; 0.1 M sodium phosphate, pH 7.0, 0.15 M NaCl, 1 mM EDTA) containing 5 mM glutathione and 1 mM diisopropylphosphorofluoridate. Hemolymph was centrifuged at 5,000 *g* for 10 min at 4°C to remove hemocytes; then the density of the solution was adjusted to 1.31 g/ml by the addition of solid KBr. HDLp-A was isolated from 1- or 2-day-old adult moths by density gradient ultracentrifugation as described by Ryan et al. (3) and HDLp-W1 and HDLp-W2 were isolated from larvae just prior to pupation according to Prasad et al. (2) and VHDLp-E was isolated from oocyte homogenates as described by Kawooya, Osir and Law (7).

Electron microscopy

Negatively stained samples (2% sodium phosphotungstate, pH 7.0) were prepared as previously described (11) and photographed in a Philips EM420 operated at 100 kV.

Circular dichroism

Circular dichroism (CD) measurements were made with a Jasco J-720 spectropolarimeter (Jasco Inc., Easton, MD) interfaced to an Epson 386/25 computer and controlled by software developed by Jasco. The cell was maintained at 25°C with an RMS circulating water bath (Lauda, Westbury, NY). In the wavelength range 255 to 190 nm, cells of path length 0.01 cm were used. The computer-averaged trace of ten scans was used in all calculations and signal due to solvent was subtracted. The instrument was routinely calibrated with d-(+)-10-camphor sulfonic acid at 290 nm, by following procedures outlined by the manufacturer. As a prerequisite to prevent distortion of the CD spectrum, at low wavelengths, the high tension voltage on the photomultiplier was never allowed to exceed 500 V.

The data were normally plotted as mean residue weight ellipticity (expressed in degrees centimeters square per decimole) versus wavelength in nm. The mean residue weight was taken to be 115 for each of the lipoproteins. Lipoprotein concentrations were determined by fringe count in the analytical ultracentrifuge (12) using an average refractive increment of 4.1 fringes per mg protein per ml. A refractive index increment of 0.178 at 546 nm was used for HDLp-W1 (13), whereas values between 0.178 and 0.185 (100% protein) were interpolated for HDLp-W2 and VHDLp-E. The concentration of the protein portion of each entity was used in the calculation of molar ellipticities. Since spectra of lipid extracts of HDLp-W1 revealed that the lipid contribution to the CD spectra was negligible, no correction was applied. The ellipticity-versus-wavelength data were analyzed by a computer program (CONTIN) developed by Provencher and Glöckner (14), which analyzes CD spectra as the sum of spectra of 16 proteins, whose structures are known from X-ray crystallography. The input to the program was the mean residue weight ellipticities, at 1 nm intervals, from 190 nm to 240 nm.

Trypsin digestion

Lipophorin samples were dialyzed into PBS and the protein concentration was adjusted to 1 mg /ml. Fifty μg of a given lipophorin was then incubated with bovine pancreatic trypsin (Boerhinger) at a trypsin: lipophorin ratio of 1:200 at 25°C for times ranging from 10 min to 1 h. Control samples were incubated in the absence of trypsin, and the reaction was stopped by placing the samples in SDS sample treatment buffer and immediately boiling for 2 min. For some experiments reactions were stopped by the addition of a twofold molar excess of soybean trypsin inhibitor (Boerhinger), with similar results. The samples

were then analyzed by electrophoresis in 4–15% acrylamide gradient slab gels in the presence of SDS (15).

Fluorescence quenching studies

Fluorescence measurements of HDLp-W1, HDLp-W2, and VHDLp-E were performed at 20°C using a Perkin–Elmer Model 44B spectrofluorometer equipped with a DCSU-2 differential corrected spectra unit and a thermostatted cell holder. Measurements were conducted with 1-ml volume Suprasil cells (Hellma) and 5-nm slit widths for both excitation and emission monochromaters. Spectra were obtained by using an excitation wavelength of 295 nm with emission monitored between 325 and 327 nm. Quenching studies were performed by the addition of aliquots of acrylamide in accordance with the methodology described by McCubbin and Kay (16). The fluorescence (F) values were corrected for acrylamide absorption using $F_{corr} = F \cdot 10^{A/2}$, where A is the absorbance in the 1-cm cell at 295 nm.

The fluorescence quenching data were handled via the Stern-Volmer equation: $F_o/F = 1 + K_{sv}[Q]$ where F_o and F represent the fluorescence intensities at the emission maximum in the absence and presence of quencher (Q). The collisional quenching constant, K_{sv} was obtained from the slope of a plot of F_o/F versus [Q].

RESULTS

At least five distinct HDLp subspecies from *M. sexta* hemolymph have been isolated and characterized (4). In addition, hemolymph and oocyte very high density lipophorins have been identified (7, 8). All HDLp subspecies, except HDLp-A, contain one molecule each of apoLp-I and apoLp-II as their sole apolipoprotein components; HDLp-A also contains two molecules of apoLp-III. The major difference between the various subspecies is in the amount of diacylglycerol per particle; this represents from 25% (HDLp-A) to 20% (HDLp-W1) to 12.5% (HDLp-W2) to 4% (VHDLp-E) of the particle mass. These values correlate with the observed buoyant densities of the individual subspecies, which range from 1.076 g/ml (HDLp-A) to 1.128 g/ml (HDLp-W1) to 1.177 g/ml (HDLp-W2) to 1.238 g/ml (VHDLp-E). The large variations in particle lipid content and density, together with the known interconvertibility of specific subspecies, indicates structural resiliency of the apolipoprotein components. A major unanswered question with respect to lipophorin relates to the effect the above-mentioned alterations in particle lipid content have on the structural properties and morphology of lipophorin.

Electron microscopy

In order to correlate compositional and physical measurements of lipophorins with proposed models of lipophorin structure, the morphologies of isolated particles were examined by electron microscopy. **Fig. 1** (panel A) reveals that the neutral lipid-deficient VHDLp-E (4% diacylglycerol) assumes an asymmetric, crescent-like conformation in which the two ends of the particle are not of uniform size. The lipophorin subspecies, HDLp-W2, which contains three times more diacylglycerol per particle (12.5%) than does VHDLp-E but has identical apolipoprotein components, also does not display a spherical morphology. This lipophorin has a U-shaped or cup-like appearance (**Fig. 1B**) and exhibits a more closed conformation than does VHDLp-E. The observed morphologies of these two subspecies demonstrate that the increased neutral lipid content of HDLp-W2 compared to VHDLp-E decreased the overall asymmetry of the particle. This trend is continued with the formation of HDLp-W1 (20% diacylglycerol) as shown in **Fig. 1C**. These particles exhibit a roughly rectangular shape with a length of 16 ± 1 nm ($n = 100$) and a width of 11 ± 1 nm. There is also a cleft that forms a distinct indentation extending from one end of the particle. When the neutral lipid content of particles is increased even further, as in HDLp-A (25% diacylglcyerol; **Fig. 1D**), a more spherical structure with a diameter of 15 ± 1 nm is formed. The particle size measured for HDLp-A is consistent with that reported for HDLps from other species (17–19). In contrast to the other HDLps, the concomitant association of two molecules of apoLp-III likely contributes to the overall sphere–like morphology of HDLp-A.

Based on these results it is conceivable that the neutral lipid–deficient, apolipoprotein/phospholipid complex referred to as VHDLp-E possesses the structural capacity to accept and stabilize increasing amounts of neutral lipids. From the observed morphologies of the different subspecies examined, it seems most likely that exogenous lipid occupies a central pocket and is surrounded by the apolipoprotein/phospholipid matrix, which can adapt its structure to accommodate varying amounts of neutral lipid. When the maximum amount of neutral lipid is reached, a spherical structure is achieved. This is consistent with the fact that additional neutral lipid can be added to HDLp-A only when there is concomitant binding of up to 16 molecules of apoLp-III (1,20).

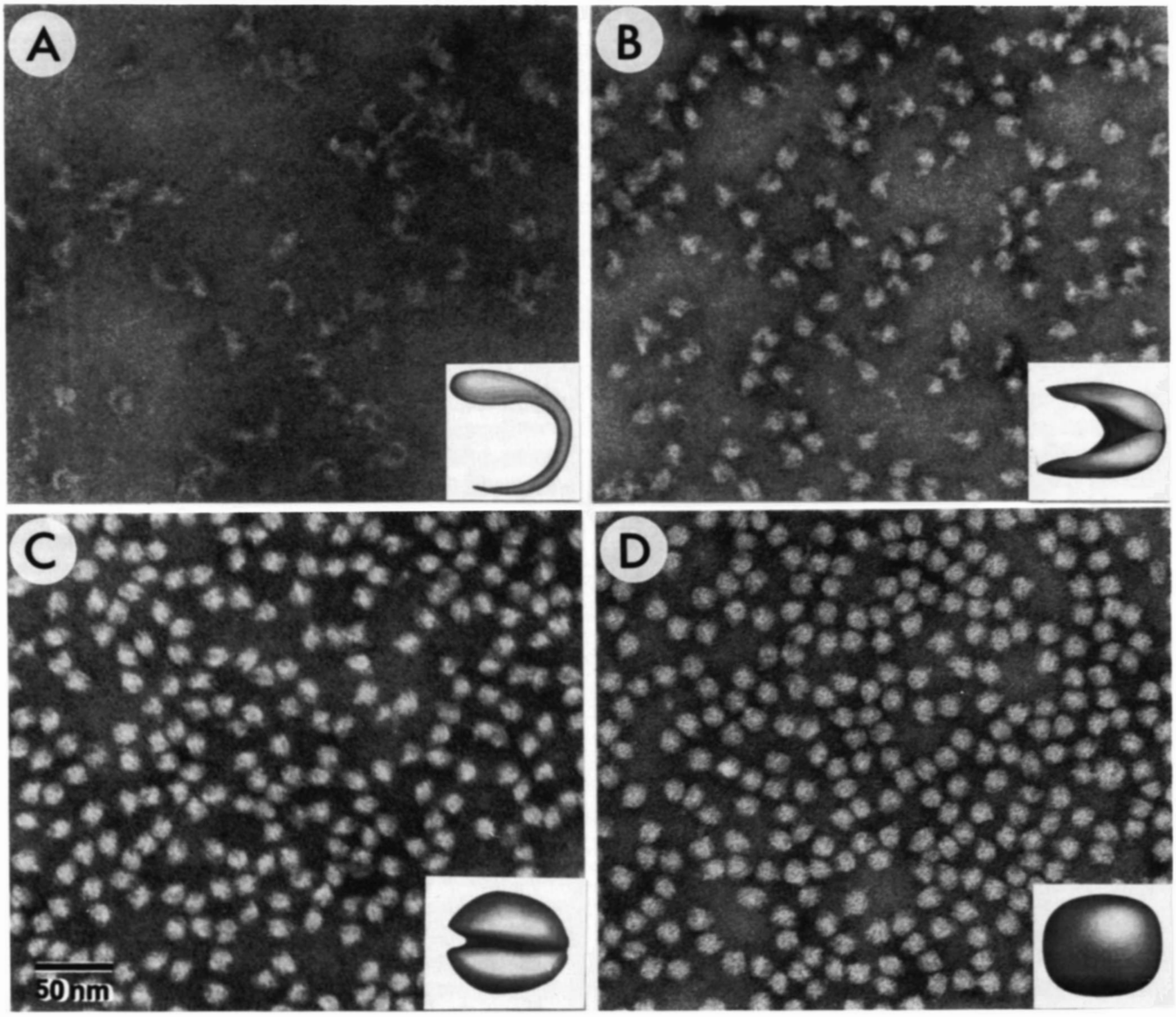

Fig. 1. Electron micrographs of lipophorin subspecies. A) VHDLp-E; B) HDLp-W2; C) HDLp-W1; D) HDLp-A. Samples (20 μg protein/ml) were negatively stained with 2% sodium phosphotungstate. Drawings, representative of the morphologies present in the respective micrographs, are shown in the lower right hand corner of each panel. It should be noted that VHDLp-E and HDLp-W2 display a degree of structural flexibility, and the drawings represent an "average" view.

Circular dichroism spectroscopy

CD experiments were conducted in order to assess whether the morphological differences observed in lipophorin structure as the neutral lipid content changes are accompanied by changes in apolipoprotein secondary structure. Spectra were obtained for HDLp-W1, HDLp-W2, and VHDLp-E (**Fig. 2**) but not for HDLp-A because, in addition to apoLp-I and apoLp-II, it contains two molecules of apoLp-III. The CD spectra for the lipophorins exhibited a minimum at 218 nm for HDLp-W1 and HDLp-W2 and at 220 for VHDLp-E, whereas the maxima occurred at 193 nm for both HDLp-W1 and HDLp-W2 and at 194 nm for VHDLp-E. The positions of the minima and the maxima are suggestive of the presence of a β-like structure for these proteins which is, in turn, reflected in the CONTIN analysis of the CD spectra (**Table 1**). While it is appreciated that these analyses are based on the CD spectra of globular proteins, and may not be entirely applicable to lipoproteins, the minor variations in the relative amounts of the various structural forms among the three lipophorins implies that only minor changes in the global protein structure occur as lipid is added or removed. In particular, HDLp-W1 and HDLp-W2 comprise essentially identical amounts of the three conformers, whereas VHDLp-E has a

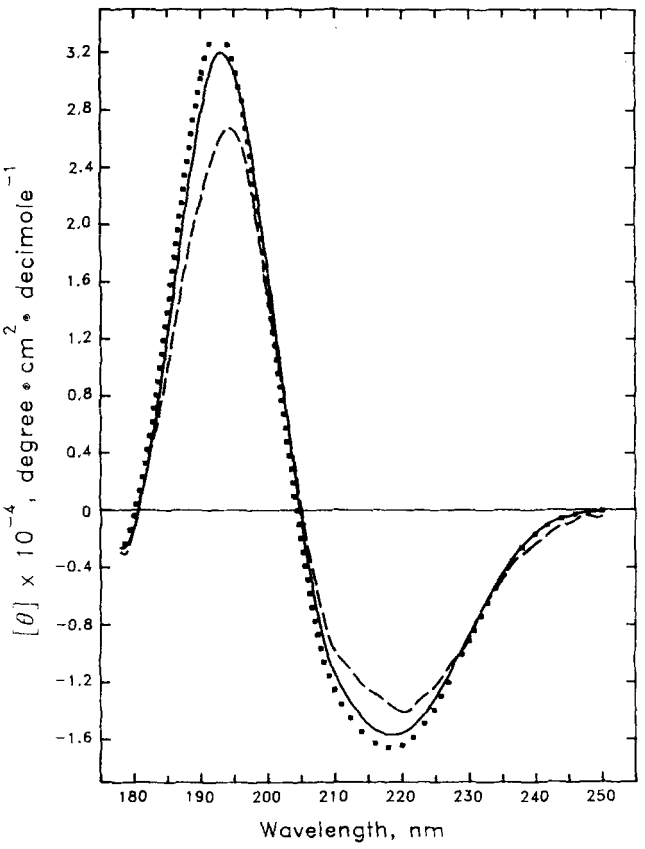

Fig. 2. Circular dichroism spectra of intact lipophorin subspecies. Solid line, HDLp-W1; closed squares, HDLp-W2; and broken line, VHDLp-E. Measurements were taken at 25°C in 50 nM sodium phosphate, pH 7.0.

comparable amount of α-helix but a reduction in β-structure with a corresponding increase in random coil.

Proteolytic digestion

It has been shown previously that apoLp-I and apoLp-II of *M. sexta* larval lipophorin display a differential susceptibility toward proteolysis by trypsin (21). Generally, apoLp-I has been observed to be proteolyzed to a much larger extent than apoLp-II in limited proteolysis experiments (22, 23). This result, along with immunological and radiolabeling evidence, has led to the suggestion that apoLp-II may not be exposed to the aqueous environment to the same extent as apoLp-I (24, 25). As an extension of this experimental approach we tested different lipophorin subspecies for apolipoprotein susceptibility to trypsin digestion. The objective of these experiments was to determine the effect of particle neutral lipid content on the degree of apolipophorin susceptibility to proteolysis. HDLp-A was not included in these experiments because it contains apoLp-III, which could also affect the accessibility of apoLp-I and apoLp-II to the enzyme. The results, shown in **Fig. 3**, revealed that, under the conditions used, the apolipoproteins in HDLp-W1 were relatively resistant to proteolysis, with only a small amount of breakdown of apoLp-I during the course of the experiment. By contrast, the HDLp-W2 and VHDLp-E apolipoproteins showed an increasing susceptibility to proteolysis. In both these particles apoLp-I was preferentially degraded to lower molecular weight components, while apoLp-II was relatively more resistant. When the amount of trypsin was increased to a 1:1 ratio, each of the substrate lipoproteins was degraded to low molecular weight products ($M_r < 35{,}000$), indicating that HDLp-W1 is not inherently trypsin-insensitive (data not shown). Taken together, the results suggest that increasing the neutral lipid content of the lipophorin particles confers apolipoprotein resistance to proteolysis by preventing the protease access to basic residues.

TABLE 1. CONTIN analysis of the CD spectra of lipophorins

Lipophorin	Percent Structural Form		
	α-Helix	β-Form	Random Coil
HDLp-W1	34	47	15
HDLp-W2	36	49	15
VHDLp-E	34	35	31

Fluorescence studies

The relative exposure of tryptophan residues to fluorescence quenching by acrylamide was determined in different lipophorin subspecies. The fluorescence emission maxima were 325 nm for HDLp-W1 and 327 for HDLp-W2 and VHDLp-E. Based on the tryptophan fluorescence maxima observed for fully solvent exposed apolipophorin I (351 nm) (26), these values indicate the presence of buried or partially buried tryptophans. It is known that 32 of the 34 tryptophan residues present in lipophorin are located in apoLp-I (24). Due to the large number of tryptophans in the molecule, an averaging effect will be noted. **Fig. 4** shows Stern-Volmer plots for HDLp-W1, HDLp-W2, and VHDLp-E. Clear differences were observed in the accessibility of tryptophan residues present in the lipophorin subspecies to quenching by acrylamide, with increasing quenching observed as the lipid content of the particles decreases. This is substantiated by the increasing K_{sv} values, derived from the slopes of the plots in Fig. 4, which yielded values of 1.254 M^{-1}, 1.558 M^{-1} and 2.744 M^{-1} for HDLp-W1, HDLp-W2 and VHDLp-E, respectively (27, 28). A reasonable interpretation of these data would be that interaction of tryptophan residues with lipid in HDLp-W1 shields these residues from the quencher,

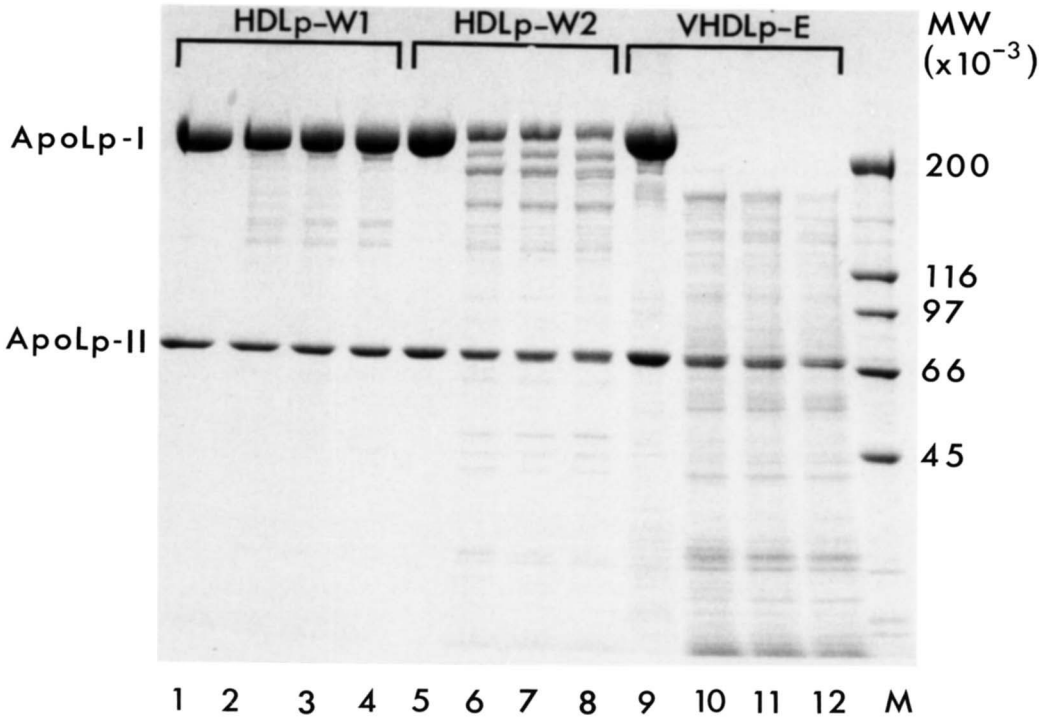

Fig. 3. Effect of trypsin on the electrophoretic pattern of lipophorin apolipoproteins. Lipophorin samples were incubated with trypsin (1:200 weight ratio) for various time at 25°C. After incubation SDS–PAGE sample treatment buffer was added and the samples were placed in boiling water bath for 2 min. The samples were then electrophoresed on a 4–15% acrylamide gradient SDS slab gel. Lanes 1–4, HDLp-W1, control, 10 min, 30 min, and 60 min, respectively. Lanes 5–8, HDLp-W2, control, 10 min, 30 min, and 60 min, respectively. Lanes 9–12, VHDLp-E, control, 10 min, 30 min, and 60 min, respectively. Lane 13, molecular weight markers.

and as the lipid content is depleted the residues become more exposed to the solvent.

DISCUSSION

One of the most intriguing aspects of the multifunctional transport lipoprotein, lipophorin, is its capacity to function as a reusable lipid shuttle (17). It is possible that hemolymph lipid transfer particle (1) plays an important role in lipophorin interconversions by mediating changes in the lipid content of lipophorin subspecies via facilitated net lipid transfer. It is generally accepted that lipophorin meets the bulk of physiological lipid transport demands without destruction of its basic matrix structure; for example, Downer and Chino (29) found that, whereas diacylglycerol has a half-life of 2–3 h in hemolymph, apoLp-I and -II have a half-life of 5–6 days. Other studies have demonstrated that lipophorin protein components are conserved while functioning to transport diacylglycerol from fat body storage depot to tissues of utilization (30). Furthermore, it has been shown that specific lipophorin subspecies are interconvertible and that naturally occurring, developmentally regulated subspecies of lipophorin arise from modification of the lipid content and composition of preexisting particles rather than from new lipophorin (or apolipophorin) biosynthesis (2). Since many of the changes in the lipid content of lipophorin subspecies occur without addition or removal of apolipoprotein, it was of interest to examine the effect of varying lipid content on the structural properties of lipophorin. While it is generally accepted that HDLp-A is a spherical particle, the effect of diacylglycerol depletion on its structural characteristics has not been examined in detail. It could be that as the neutral lipid content of lipophorin decreases, other components, such as apoLp-II, reorient to occupy a greater portion of the particle core with maintenance of an overall spherical structure. Alternatively, loss of neutral lipid from the core of the particle could generate nonspherical entities.

The data reported in the present paper supports the latter alternative and has important implications with respect to the development of structural models for lipophorin. In subspecies other than HDLp-A, a spherical structure cannot be assumed; in fact, as the density of the subspecies increases (and lipid content decreases) there is a progressive deviation from a

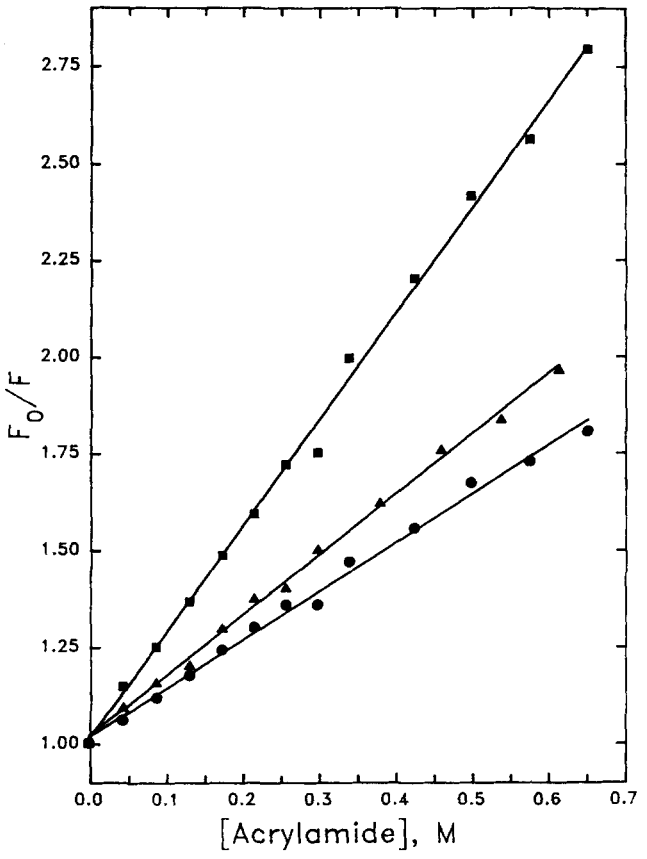

Fig. 4. Stern-Volmer plots of acrylamide fluorescence quenching of lipophorin subspecies. Closed squares, VHDLp-E; closed triangles, HDLp-W2, and closed circles, HDLp-W1. Solutions of lipophorin in PBS were excited at 295 nm and fluorescence emission was monitored at 325 nm (HDLp-W1) or 327 nm (HDLp-W2 and VHDLp-E). The concentration of acrylamide in the samples was increased by the addition of aliquots of 8M acrylamide.

spherical structure. The observed changes in morphology have been correlated with changes in apolipoprotein susceptibility to proteolysis as well as with the relative exposure of tryptophan residues to fluorescence quenching by acrylamide. If the lipophorin particle maintained a spherical structure as it lost neutral lipid, an increasing proportion of apoLp-I and/or apoLp-II would be required to replace lipid in the core of the particle. This would result in a particle of smaller diameter but with an overall decrease in the amount of apolipoprotein exposed to the aqueous environment. Contrary to this prediction, we observed a dramatic increase in apolipoprotein susceptibility to proteolysis and tryptophan fluorescence quenching as the neutral lipid content of the particle was decreased. These results support the conclusions drawn from the electron micrographs that suggest an apolipoprotein/phospholipid framework encloses a central cavity to which lipid can be added or from which it can be removed. This structural arrangement could conceivably be designed to stabilize the lipid-depleted basic matrix structure or to expose cryptic membrane docking or receptor sites (31, 32) so that intracellular lipid stores can be accessed.

The results of CD spectroscopy on HDLp-W1 and HDLp-W2 are in general agreement with results previously reported for lipophorins (23, 33) although the CONTIN analysis suggests the proteins contain 10–15% less β-structure. VHDLp-E contained a decreased amount of β-structure and an increase in random coil compared to HDLp-W2 and HDLp-W1 but had a similar amount of α-helix. The increase in random coil at the expense of β-structure, together with its low content of neutral lipid and asymmetric morphology, suggests that the overall secondary structure of lipophorin is influenced by its lipid component. The relatively small changes in CD indicate that the framework does not reorient to any major extent, and suggest that increases in proteolytic susceptibility and tryptophan fluorescence quenching reflect the exposure of protein caused by emptying the interior cavity of neutral lipid.

In a study of the transformation of HDLp-A to VHDLp-E in *M. sexta* oocytes, Kawooya, Osir, and Law (7), reported that VHDLp-E has a smaller size and elutes later than HDLp-A when subjected to gel permeation chromatography. This conclusion assumes that both particles are spherical. However, the electron micrographs of VHDLp-E show an asymmetric object with obvious structural flexibility. When taken together with its ability to reversibly bind lipid (17), the data suggest that VHDLp-E should not be viewed as a rigid structure but rather as an apolipoprotein/phospholipid framework that exhibits orientational freedom compared to relatively lipid-enriched lipophorin subspecies. This is likely due to the fact that VHDLp-E generally lacks neutral core lipids that require sequestration from the aqueous environment, thereby imposing restrictions on the apolipoprotein/phospholipid framework flexibility. The present results regarding the morphology of VHDLp-E are not in agreement with an earlier report by Chino, Downer, and Takahaski (34), who studied hemolymph and egg lipophorins of the silkworm *Philosamia cynthia*. These investigators reported that HDLp-A and VHDLp-E displayed essentially the same morphology, although VHDLp-E was largely depleted of lipid. They offered no explanation as to why these two particles would have the same diameter in spite of their substantial differences in lipid content. In contrast, we have shown a dramatic change in lipophorin morphology consistent with differences in lipid composition. In a study of larval lipophorin of *M. sexta* (1.15 g/ml), which contains the same two apolipoproteins and an overall lipid content inter-

mediate between HDLp-W1 and HDLp-W2, Pattnaik et al. (21) also used electron microscopy. Although these workers did not differentiate among different subspecies, micrographs of their preparation exhibited significant heterogeneity and contained many objects which resemble our HDLp-W1 and HDLp-W2 particles.

The morphologies of HDLp-W2 and VHDLp-E are different from those of mammalian lipoproteins, which are spherical in all classes ranging from high density lipoprotein to chylomicrons. Lipoproteins found in the hemolymph of Crustacea such as the spiny lobster, *Panulirus interruptus* (35) or the crab, *Cancer antennarius* (36), however, are polymorphic. Some particles are similar in appearance to HDLp-W2 or VHDLp-E. Crustacean high density lipoprotein particles are similar to the diacylglycerol-poor lipophorin subspecies in that they are relatively protein- and phospholipid-rich and the protein components are of high molecular weight (84,000–185,000 daltons). The significance of this observation with respect to lipoprotein evolution has yet to be assessed, but it is tempting to speculate that specialized physiological lipid transport needs of insects that arose during evolution (i.e., flight) may have resulted in an adaptation whereby circulating lipoproteins could be modified to accept more neutral lipid resulting in formation of more sphere-like structures. The advantage of such a system is that it permits lipid mobilization without new lipoprotein biosynthesis and results in an increased speed and efficiency of lipid transport processes.

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REFERENCES

1. Ryan, R. O. 1990. The dynamics of insect lipophorin metabolism. *J. Lipid Res.* **31:** 1725–1739.
2. Prasad, S. V., R. O. Ryan, J. H. Law, and M.A. wells. 1986. Changes in lipoprotein composition during larval-pupal metamorphosis of an insect *Manduca sexta*. *J. Biol. Chem.* **261:** 558–562.
3. Ryan, R. O., S. V. Prasad, E. J. Henrickson, M. A. Wells, and J. H. Law. 1986. Lipoprotein interconversions in an insect, *Manduca sexta*. Evidence for a lipid transfer factor in the hemolymph. *J. Biol. Chem.* **261:** 563–568.
4. Shapiro, J. P., J. H. Law, and M. A. Wells. 1988. Lipid transport in insects. *Annu. Rev. Entomol.* **33:** 297–318.
5. Kawooya, J. K., P. S. Keim, R. O. Ryan, J. P. Shapiro, P. Samaraweera, and J. H. Law. 1984. Insect apolipophorin III. Purification and properties. *J. Biol. Chem.* **259:** 10733–10737.
6. Cole, K. D., G. J. P. Fernando-Warnakulasuriya, M. S. Boguski, M. Freeman, J. I. Gordon, and W. A. Clark. 1987. Primary structure and comparative sequence analysis of an insect apolipoprotein: apolipophorin III from *Manduca sexta*. *J. Biol. Chem.* **262:** 11794–11800.
7. Kawooya, J. K., E. O. Osir, and J. H. Law. 1988. Uptake of the major hemolymph lipoprotein and its transformation in the insect egg. *J. Biol. Chem.* **263:** 8740–8747.
8. Prasad, S. V., G. J. P. Fernando-Warnakulasuriya, M. Sumida, J. H. Law, and M. A. Wells. 1986. Lipoprotein biosynthesis in the larvae of the tobacco hornworm, *Manduca sexta*. *J. Biol. Chem.* **261:** 17174–17176.
9. Shen, B. W., A. M. Scanu, and F. J. Kézdy. 1977. Structure of human serum lipoproteins inferred from compositional analysis. *Proc. Natl. Acad. Sci. USA.* **74:** 837–841.
10. Soulages, J. L., and R. R. Brenner. 1991. Study of the composition–structure relationship of lipophorins. *J. Lipid Res.* **32:** 407–415.
11. Ryan, R. O., A. Howe, and D. G. Scraba. 1990. Studies of the morphology and structure of the plasma lipid transfer particle from the tobacco hornworm, *Manduca sexta*. *J. Lipid Res.* **31:** 871–879.
12. Babul, J., and E. Stellwagen. 1969. Measurement of protein concentration with interference optics. *Anal. Biochem.* **28:** 216–221.
13. Handbook of Biochemistry and Molecular Biology. 1975. G. D. Fasman, editor. CRC Press, Cleveland.
14. Provencher, S. W., and J. Glöckner. 1981. Estimation of globular protein secondary structure from circular dichroism. *Biochemistry.* **20:** 33–37.
15. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature.* **227:** 680–685.
16. McCubbin, W. D., and C. M. Kay. 1980. Acrylamide fluorescence quenching studies on skeletal and cardiac troponins. *FEBS Lett.* **122:** 72–76.
17. Chino, H. 1985. Lipid transport: biochemistry of hemolymph lipophorin. *In* Comprehensive Insect Physiology, Biochemistry and Pharmacology. G.A. Kerkut and L.I. Gilbert, editors. Pergamon Press, Oxford. **10:** 115–136.
18. Van Antwerpen, R., W. A. M. Linnemans, D. J. Van der Horst, and A.M.T. Beenakkers. 1988. Immunocytochemical localization of lipophorins in the flight muscle of the migrating locust *(Locusta migratoria)* at rest and during flight. *Cell Tissue Res.* **252:** 661–668.
19. Wheeler, C. H., J. E. Mundy, and G. J. Goldsworthy. 1984. Locust haemolymph lipoproteins visualised in the electron microscope. *J. Comp. Physiol.* **154:** 281–286.
20. Wells, M. A., R. O. Ryan, J. K. Kawooya, and J. H. Law. 1987. The role of apolipophorin III in in vivo lipoprotein interconversions in adult *Manduca sexta*. *J. Biol. Chem.* **262:** 4172–4176.
21. Pattnaik, N. M., E. C. Mundall, B. G. Trambusti, J.H. Law, and F.J. Kézdy. 1979. Isolation and characterization of a larval lipoprotein from the hemolymph of *Manduca sexta*. *Comp. Biochem. Physiol.* **63B:** 469–476.
22. Robbs, S. L., R. O. Ryan, J. O. Schmidt, P. S. Keim, and J. H. Law. 1985. Lipophorin of the larval honeybee, *Apis mellifera*. *J. Lipid Res.* **26:** 241–247.

23. Kashiwazaki, Y., and A. Ikai. 1985. Structure of apoproteins in insect lipophorin. *Arch. Biochem. Biophys.* **237:** 160–169.
24. Shapiro, S. P., P. S. Keim, and J. H. Law. 1984. Structural studies on lipophorin: an insect lipoprotein. *J. Biol. Chem.* **259:** 3680–3685.
25. Mundall, E. C., N. M. Pattnaik, B. G. Trambusti, G. Hromnak, F. J. Kézdy, and J. H. Law. 1980. Structural studies on an insect high density lipoprotein. *Ann. N.Y. Acad. Sci.* **348:** 431–432.
26. Soulages, J. L., O. J. Rimoldi, and R. B. Brenner. 1988. Lipid thermotropic transitions in *Triatoma infestans* lipophorin. *J. Lipid Res.* **29:** 172–182.
27. Eftink, M. R., and C. A. Ghiron. 1976. Exposure of tryptophanyl residues in proteins. Quantitative determination by florescence quenching studies. *Biochemistry.* **15:** 672–680.
28. Eftink, M. R., and C. A. Ghiron. 1977. Exposure of tryptophanyl residues and protein dynamics. *Biochemistry.* **16:** 5546–5551.
29. Downer, R. G. H., and H. Chino. 1985. Turnover of protein and diacylglycerol components of lipophorin in locust hemolymph. *Insect Biochem.* **15:** 627–630.
30. Van Heusden, M. C., D. J. Van der Horst, J. Voshol, and A.M.T. Beenakkers. 1987. The recycling of protein components of the flight-specific lipophorin in *Locusta migratoria*. *Insect Biochem.* **17:** 771–776.
31. Van Antwerpen, R., H. J. A. Wynne, D. J. Van der Horst, and A. M. T. Beenakkers. 1989. Binding of lipophorin to the fat body of the migratory locust. *Insect Biochem.* **19:** 809–814.
32. Tsuchida, K., and M. A. Wells. 1990. Isolation and characterization of a lipoprotein receptor from the fat body of an insect, *Manduca sexta*. *J. Biol. Chem.* **265:** 5761–5767.
33. Kawooya, J. K., M. A. Wells, and J. H. Law. 1989. A strategy for solubilizing delipidated apolipoprotein with lysophosphatidylcholine and reconstitution with phosphatidylcholine. *Biochemistry.* **28:** 6658–6667.
34. Chino, H., R. G. H. Downer, and K. Takahashi. 1977. The role of diacylglycerol–carrying lipoprotein–I in lipid transport during insect vitellogenesis. *Biochim. Biophys. Acta.* **487:** 508–516.
35. Lee, R. F., and D. L. Puppione. 1978. Serum lipoproteins in the spiny lobster *Panulirus interruptus*. *Comp. Biochem. Physiol.* **59B:** 239–243.
36. Spaziani, E., R. J. Havel, R. L. Hamilton, J. B. Hardman, J. B. Stoudemire, and R. D. Watson. 1986. Properties of serum high–density lipoproteins in the crab, *Cancer antennarius* Stimpson. *Comp. Biochem. Physiol.* **85B:** 307–314.