

LIPID TRANSPORT IN INSECTS¹

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PERSPECTIVES AND OVERVIEW

"Oil and water do not mix," a fact of our common observation, is a problem that must be circumvented in living organisms. Since the oily or fatty materials are generally produced or stored at one location in the organism and used or deposited in a different location, a device is necessary to move these hydrophobic materials through an aqueous environment. In animals a partial solution to this problem is provided by emulsifying agents that disperse fats and oils in the form of small droplets so they may be digested in the gut, prior to absorption. Unfortunately, these agents, akin to synthetic laundry detergents, cannot be used in the blood because they are capable of disrupting cell membranes. Therefore, animals developed a different transport vehicle for moving hydrophobic materials through the blood, the lipoprotein.

Much is now known about the structure and function of the diverse array of mammalian lipoproteins. In general, the lipoprotein consists of a nonpolar spherical core composed of cholesterol esters and triacylglycerols, surrounded by a monolayer of polar phospholipids and cholesterol as well as a coating of proteins called apolipoproteins. It is important to distinguish apoproteins of lipoproteins from subunits of oligomeric proteins. The latter subunits have a definite stoichiometry and geometrical relationship to one another. They are rigidly held in place by multiple contacts involving ionic and hydrogen bonds,

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as well as by hydrophobic interactions. In lipoproteins, the lipids and apoproteins are held together largely by the hydrophobic effect (the apoproteins may not even contact one another) and their geometric relationship may not be rigidly defined. If lipids are removed, the resulting apoproteins may have no affinity for one another and will frequently be insoluble in water. The polar head groups of lipids (including cholesterol) interact with the polar groups of the proteins and with water, while the hydrophobic portions of the proteins interact with the nonpolar fatty acyl chains and cholesterol. This provides a water-soluble package, which is hydrated and charged on the outside and oily on the inside.

In insects the lipid transport problem appears to be solved in similar ways, by the construction of lipoproteins. It appears that insect lipoproteins are less diverse but more versatile and efficient than their mammalian analogs. Mammals have several quite different lipoproteins [e.g. chylomicrons, very-low-density lipoproteins (VLDL)², low-density lipoproteins (LDL), high-density lipoproteins (HDL), and very-high-density lipoproteins (VHDL)], some of which are taken up by cells and destroyed in the course of transporting lipids. Insects, however, appear to rely on a single type of lipoprotein (lipophorin) for most lipid transport. Insect lipophorin seems to be composed of a basic matrix containing two apolipoproteins and a complement of mostly polar lipids, to which an additional apolipoprotein and more lipids can be added as special needs demand. Lipophorin generally functions as a reusable shuttle for lipids, and does not appear to be taken up or degraded during its functioning. Lipophorin moves digested fat from the gut to tissues for cell membrane construction, to muscle for combustion, or to or from storage sites. It has additional functions in the distribution of hydrocarbons, cholesterol, and carotenoids, and seems to be involved in the distribution of hydrophobic xenobiotics. In some insects, lipophorin appears to be intimately involved in hemolymph clotting reactions.

While lipophorin appears to be common to all insects so far examined, other specialized lipoproteins have also been identified. Foremost among these is the egg yolk protein precursor, vitellogenin, which is a VHDL.

Two excellent reviews of research on insect lipoproteins have recently been published (2, 7). Beenackers et al (2) described the early history of lipophorin research in detail. We begin our review with a brief summary to bring the reader up to date, then review recent progress in this fast-moving field, and finally suggest some directions for future research.

²Lipoproteins are conveniently classified by density. The presence of differing amounts of low-density lipids results in a range of densities, all less than those of nonlipoproteins. Density is easily determined by centrifugal methods.

BRIEF HISTORY

Before proceeding, it is important to define the nomenclature used. Lipoproteins are named according to their density class. The larval forms and those found in resting adults are of the high-density class, and are called high-density lipophorin or HDLp. The forms that carry large amounts of lipid from the fat body of the adult to flight muscle are of low density and are called low-density lipophorin or LDLp.

The three apolipoproteins are called apoLp-I (~250 kd), apoLp-II (~80 kd) and apoLp-III (~18–20 kd). Early studies of lipid transport in insect hemolymph revealed that phospholipids and diacylglycerols are major lipid components (11, 12, 83, 89). This fact sets the insects apart from mammals, which have high levels of triacylglycerols in the blood while diacylglycerols are minor components. Diacylglycerols are released from the fat body in vitro and associate with protein components of the hemolymph (11, 12, 89). Thomas & Gilbert (88) isolated lipoproteins from *Hyalophora cecropia* hemolymph by density gradient centrifugation and separated them into LDL, HDL, and VHDL classes. Chino et al (16, 18) isolated two diacylglycerol-rich lipoproteins from *Philosamia cynthia* hemolymph. One of these, a VHDL, was subsequently shown to be female specific and identical to vitellogenin (19). The other lipoprotein, a HDL, was later given the name lipophorin (10) in recognition of its function as a reusable lipid shuttle vehicle (24). This role of lipophorin was suggested by the demonstration that it could take up lipids (sterols and diacylglycerols) derived from digestion of foods in the midgut (13, 16). The composition of the well-characterized lipophorins is presented in Table 1.

The shuttle function of lipophorin was more clearly defined when its role in lipid transport from fat body to flight muscle in certain groups of flying insects was described. Mwangi & Goldsworthy (56), studying flight metabolism in the locust *Locusta migratoria*, showed that lipophorin increased in size either during flight or upon injection of extracts of the corpus cardiacum (57). Similar results have been presented for *Manduca sexta* (114). The corpus cardiacum extracts were shown to contain a decapeptide hormone, the adipokinetic hormone (AKH) (1, 86), which was responsible for lipid mobilization. Using *L. migratoria*, van Heusden et al (100) carried out definitive experiments that showed that lipophorin could accept diacylglycerol from fat body in vitro under the influence of AKH. The low-density lipophorin thus formed could deliver diacylglycerol to flight muscle in vitro, and in the process it was converted back to a high-density lipophorin.

Wheeler & Goldsworthy (106) observed the association of a soluble hemolymph protein (termed the C fraction, now called apoLp-III) with HDLp during the addition of diacylglycerol at the fat body. The LDLp formed

Table 1 Composition (wt%) and density of insect lipophorins^a

	PR	PL	DG	HC	CH	TG	Density (g ml ⁻¹)	Ref
HDLp								
<i>Manduca sexta</i>								
Larvae	62.7	16.7	15.7	2.8	1.2	1.1	1.151	(65)
Prepupae-1	53.1	23.3	20.2	1.6	1.8	1.1	1.128	(65)
Prepupae-2	65.2	18.9	12.5	0.5	1.8	1.0	1.177	(65)
Pupae	53.6	21.6	17.5	0.4	2.8	1.0	1.139	(65)
Adults	48.5	14.0	25.0	3.5	1.3	2.5	1.076	(76)
<i>Apis mellifera</i>	59.0	12.8	13.3	2.0	6.0	3.9	1.13	(72)
<i>Locusta migratoria</i>	59.0	14.8	13.4	8.7	3.2	0.7	1.12	(15)
<i>Periplaneta americana</i>	50.0	21.4	7.6	14.2	2.5	1.0	—	(15)
<i>Phylosamia cyntia</i>	56.0	11.4	24.8	0.6	5.8	0.5	—	(15)
<i>Diatraea grandiosella</i>	62.0	11.0	13.0	—	5.4	2.7	1.11	(23)
LDLp								
<i>Manduca sexta</i>	37.8	7.1	46.9	2.3	0.7	1.7	1.03	(76)
<i>Locusta migratoria</i>	53.7	10.9	26.1	6.4	2.4	0.5	1.065	(9)

^aPR, protein; PL, phospholipid; DG, diacylglycerol; HC, hydrocarbon; CH, cholesterol; TG, triacylglycerol.

contained several molecules of apoLp-III. When the lipophorin delivered diacylglycerol to the flight muscle, apoLp-III was released as diacylglycerol was unloaded. Shapiro & Law (79) observed similar events in *M. sexta*.

LIPOPHORIN BIOSYNTHESIS

Until recently only fragmentary data were available concerning the biosynthesis of lipophorin (28, 33, 62, 87). These reports suggested that the fat body was the site of biosynthesis, but did not characterize the process. Prasad et al (64) have described, in detail, the biosynthesis of lipophorin in feeding fifth instar larvae of *M. sexta*. In vitro, the fat body made and secreted a nascent VHDLP particle that contained apoLp-I, apoLp-II, and phospholipid, but very little diacylglycerol. Prasad et al (64) suggested that formation of the mature hemolymph lipoprotein involves uptake of diacylglycerol derived from dietary lipid in the midgut. The maturation process apparently occurs in the hemolymph.

Prasad et al (64) confirmed that dietary lipid is the source of lipophorin diacylglycerol by showing that insects raised on a fat-free diet contained a circulating lipoprotein essentially devoid of diacylglycerol. This lipoprotein had a density comparable to that of the nascent secreted lipoprotein. Although the exact mechanism is unknown, the transfer of diacylglycerol from the

midgut to the nascent lipoprotein was proposed to involve a lipid transfer protein (76, 77). A schematic representation of lipophorin biosynthesis in larval *M. sexta* is shown in Figure 1.

Lipophorin biosynthesis in diapausing larvae of the southwestern corn borer (*Diatraea grandiosella*) also takes place in the fat body (101, 102). However, in this case the lipoprotein secreted by the fat body *in vitro* has a density and lipid composition similar to that of the circulating lipoprotein. It is possible that the secreted lipophorins of *M. sexta* and *D. grandiosella* differ because the diapausing *D. grandiosella* larvae do not feed and must use fat body lipid for fuel (91). Nothing is known at present about the process of intracellular assembly of the nascent lipoprotein in the larval fat body.

The biosynthesis of lipophorin during larval development in *M. sexta* does not occur continuously, but only during periods of feeding. When the amount of lipophorin per insect was measured from the beginning of the fourth instar through pupation, total lipophorin increased during the first two days of the fourth instar, remained constant during larval ecdysis, increased again during the first three days of the fifth instar, and then remained constant from the prepupal period through pupal ecdysis (66, 90). These cyclic changes were due to changes in the amounts of mRNA available for apoprotein synthesis, as measured by *in vitro* translation. The data show the presence of mRNA during the feeding stages and the absence of mRNA after cessation of feeding. The shutoff of biosynthesis corresponds to the appearance of ecdysone in the hemolymph both at the end of the fourth instar and then at the commencement

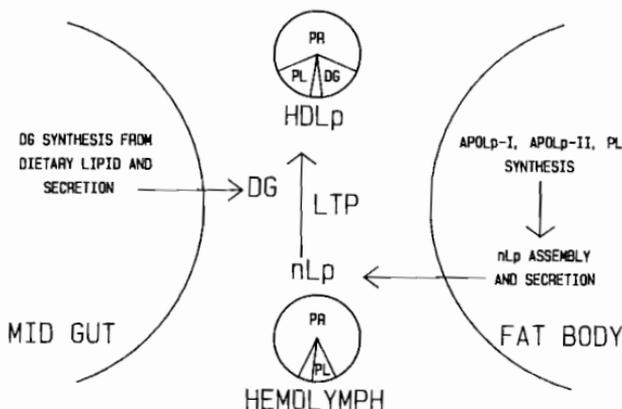


Figure 1 Biosynthesis of lipophorin in *Manduca sexta* larvae, showing final assembly in the hemolymph (64). Apoproteins are synthesized in the fat body, combined with phospholipid, and secreted into the hemolymph as a nascent particle (nLp). Diacylglycerol (DG) is derived from dietary fat in the midgut. The possible role of the hemolymph lipid transfer protein (LTP) (76, 77) in moving DG from the midgut to the nLp is indicated.

of the prepupal period. This pattern of cyclic synthesis of apolipoproteins corresponds exactly to the pattern reported for the major storage protein, arylphorin (71), as well as to those of two cuticular proteins (70). As was the case for arylphorin (71), no evidence was obtained that juvenile hormone had any role in controlling lipophorin biosynthesis (66). At present, there is no direct evidence that ecdysone or any other hormone related to feeding is involved in this gene regulation.

In the holometabolous insect *M. sexta*, lipoproteins are not biosynthesized during the pupal stage, and mRNAs for apoprotein synthesis are absent from the fat body. However, approximately 12–24 hr before adult eclosion, the apoprotein mRNAs reappear in the fat body and active synthesis of lipophorin commences (K. D. Cole & M. A. Wells; S. V. Prasad & M. A. Wells, unpublished information). The adult form of lipophorin differs from the larval and pupal forms in that it contains two molecules of apoLp-III in addition to one molecule each of apoLp-I and -II (48, 79, 103). These two molecules of apoLp-III appear to be intimately integrated into the structure of the adult lipoprotein, since they do not exchange with free apoLp-III (103). Therefore, it seems that the pupal lipophorin is replaced late in development by a newly synthesized lipophorin that contains apoLp-III. The mechanism by which this may occur is unknown at present. In the hemimetabolous insect *L. migratoria*, both the resting adult and larval lipoproteins contain only apoLp-I and -II (20), but detailed studies on the developmental profile of biosynthesis have not been reported.

As discussed in detail elsewhere in this review, the use of lipid to fuel flight is intimately associated with the presence of free apoLp-III in the hemolymph. In *L. migratoria* apoLp-III is synthesized in the fat body (40, 41). Low levels of apoLp-III are found in larval hemolymph, and the rate of synthesis increases after adult ecdysis (41), regulated in part by juvenile hormone (M. R. Kanost & G. R. Wyatt, unpublished information). Similarly, *M. sexta* larvae have low levels of apoLp-III in the hemolymph, and the levels are much higher in adult hemolymph (48). At present there is no known function for apoLp-III during larval development. Despite its synthesis in the fat body it is not incorporated into the larval lipoprotein. It is also not known what determines whether newly synthesized apoLp-III will be incorporated into the adult lipophorin or secreted free into the hemolymph.

VARIATION OF LIPOPHORIN COMPOSITION AND FUNCTION WITH DEVELOPMENT

Prasad et al (65) reported that significant changes in lipid composition and in the physical properties of lipophorin occur during metamorphosis from larva to pupa in *M. sexta*. Thus at the end of the fifth instar and at the initiation of

the prepupal stage there is a decrease in lipid content, which is followed 12 hr later by a large increase. The lipid-rich lipophorin remains in the hemolymph until pupal ecdysis, when yet another change in the lipid composition of lipophorin takes place. All of these species of lipophorin have a characteristic density and lipid composition. The changes all occur without the synthesis of apoproteins; indeed, they occur after the mRNAs that would have been used for apoprotein synthesis have disappeared from the fat body.

Although there is no synthesis or secretion of new lipophorin molecules from the fat body during these changes, the fat body is nevertheless the source of the lipid added to the hemolymph lipoprotein. For example, animals raised on a fat-free diet contain a very-high-density lipophorin throughout larval development. Nonetheless, during the prepupal stage they produce a lipophorin comparable in density and lipid composition to that of animals raised on a normal diet. Apparently, fat-body lipid stores are derived from carbohydrate during larval development (G. J. P. Fernando-Warnakulasuriya, K. Tsuchida & M. A. Wells, unpublished information).

Recently, Tsuchida et al (90) demonstrated changes in lipophorin lipid composition and density during ecdysis from the fourth to the fifth instar. Like larval-pupal changes, the changes during larval-larval ecdysis occur while apoprotein mRNAs are absent from the fat body, and therefore cannot involve synthesis and secretion of new lipophorin molecules from the fat body. It is not yet clear in this case whether the fat body or the midgut is the source of the lipid taken up by lipophorin.

The changes in the lipid content of lipophorin during both larval-larval and larval-pupal ecdysis correspond with increases in ecdysone titers in the hemolymph (65, 90). However, a direct effect of ecdysone on fat body metabolism has yet to be demonstrated.

Ryan & Law (75) suggested that the role of lipophorin during larval development is to deliver lipid, predominately diacylglycerol, to growing tissues and to the fat body for storage. In support of this conclusion, when lipid-labeled lipophorin was injected into actively feeding fifth instar larvae of *M. sexta*, the label was rapidly incorporated into fat body (K. Tsuchida & M. A. Wells, unpublished information). However, the normal development of *M. sexta* and *Galleria mellonella* (22) larvae on a fat-free diet supplemented with small amounts of cholesterol and linolenic acid shows that such a function is not essential.

Chino et al (17) have presented results that suggest that lipophorin is the source of cholesterol for ecdysone biosynthesis by the prothoracic gland. If this is true, the very-high-density lipoprotein produced on a fat-free diet apparently carries sufficient cholesterol to support ecdysone synthesis, since molting is normal.

It has also been suggested that lipophorin may have a role in transport of

hydrocarbons to the cuticle (45, 46, 65). The lipophorin produced on a fat-free diet does contain some hydrocarbons, albeit reduced amounts. It has not yet been determined whether the amount or composition of cuticle hydrocarbons is altered in insects raised on a fat-free diet.

LIPOPROTEIN EVOLUTION

All insect lipophorins that have been examined to date, including representatives from seven orders (74), have basically the same properties. Except for the low-density lipoproteins found during flight, all have molecular mass between 500 and 800 kd; densities between 1.09 and 1.15 g ml⁻¹; lipid contents between 35 and 50%, with phospholipid and diacylglycerol as the major lipids; and two apolipoproteins, apoLp-I (~250 kd) and apoLp-II (~80 kd). Except during flight, and excluding vitellogenins, this high-density lipoprotein is usually the only lipoprotein species present in insect hemolymph. Haunerland & Bowers (36) have shown that the millipede *Orthoporus ornatus* and the centipede *Scolopendra heros*, which are both from the same arthropod subphylum as insects (Uniramia), have hemolymph lipoproteins similar to those of insects. It was also shown that several species of the class Arachnida, subphylum Chelicerata, including several members of the order Araneae (*Eurypeima californicum*, tarantula; *Olios fasciculatus*, crab spider; *Latrodectus hesperus*, black widow) and one member of the order Solifugae (*Eremobates* sp., wind scorpion) have hemolymph lipoproteins similar to those of insects (N. H. Haunerland & W. S. Bowers, unpublished information). In contrast, the few species of the subphylum Crustacea that have been examined, including the terrestrial isopod *Armadillidium* sp. (N. H. Haunerland & W. S. Bowers, unpublished information) and the marine decapods *Panulirus interruptus* (spiny lobster) (52) and *Cancer antennarius* (rock crab) (67, 84) have a high-density lipoprotein (~300 kd) that contains predominately phospholipid and a single apolipoprotein (~100 kd). It is interesting to note that mites, although arachnids, have lipoproteins that are similar to those of crustaceans (85; N. H. Haunerland & W. S. Bowers, unpublished information). No reports have appeared describing lipoproteins from annelids, mollusks, or other more primitive invertebrates.

There are presently insufficient data to permit reasonable speculation on the evolutionary relationship between arthropod and vertebrate lipoproteins. Certainly, even the most primitive vertebrate examined, the hagfish, has lipoproteins more closely akin to those of mammals than to those of any arthropod (6). An examination of lipoprotein evolution based not only on the properties of the circulating lipoproteins but also on sequence comparisons of the apolipoproteins and gene structure would be an area for fruitful investigation. For example, there is little sequence identity between apoLp-III from *M. sexta*

and mammalian apolipoproteins, although there is considerable homology based on a common motif of a repeating amphiphilic unit (21). This may suggest that convergent evolution could produce apolipoproteins with common physical properties. In addition, there is no obvious explanation as to why insects transport diacylglycerols and mammals triacylglycerols.

STRUCTURAL ORGANIZATION OF LIPOPHORINS

The hydrophobic core model of mammalian lipoprotein structure (25, 80) is widely accepted. The core structure is composed of triacylglycerols and cholesterol esters. The core is surrounded by a monolayer of phospholipid and cholesterol, which serves as the amphiphilic connection between the core and the protein-water surface of the particle. In considering the application of this model to lipophorins, we relied on the facts that electron microscopy shows that lipophorins are nearly spherical particles (9, 14, 61) and that NMR data show that hydrocarbons are core components while phospholipids are surface components (43, 44). A hypothetical model for the composition of the core and surface lipids can be deduced as follows. First the volume and radius of the particle are calculated based on its molecular mass and density. Then the total volume of the protein components is subtracted to determine the volume of the lipid components. From the volume of the lipid components the radius of the lipid portion of the lipophorin is calculated. If it is assumed that the amphiphilic surface monolayer is 20.5 Å thick (25, 80), then the radius and hence the volume of the hydrophobic core can be calculated. The composition of the core is then determined by summing up the contributions of first the most hydrophobic component, hydrocarbon, and then, in the following order, triacylglycerol, cholesterol, and diacylglycerol until the core volume has been accounted for. The remaining diacylglycerol, free fatty acid (if present), and phospholipids then comprise the surface monolayer and should be present in sufficient quantity to cover the surface.

Figure 2 schematically illustrates the distribution of lipids between the core and surface layer for HDLp and LDLp from *M. sexta* and *L. migratoria*. In all cases, the surface layer contains diacylglycerol, which should facilitate movement of diacylglycerol from lipophorin to its target tissue. In *M. sexta*, diacylglycerol also constitutes a significant portion of the core of both HDLp and LDLp, whereas in *L. migratoria* the core of HDLp is made up entirely of hydrocarbon and triacylglycerol, although LDLp has a significant amount of diacylglycerol in the core. We have calculated a similar distribution for the larval, prepupal, and pupal lipophorins of *M. sexta* and have generally concluded that diacylglycerol and phospholipid make up the surface layer and that hydrocarbon, triacylglycerol, cholesterol, and diacylglycerol make up the core.

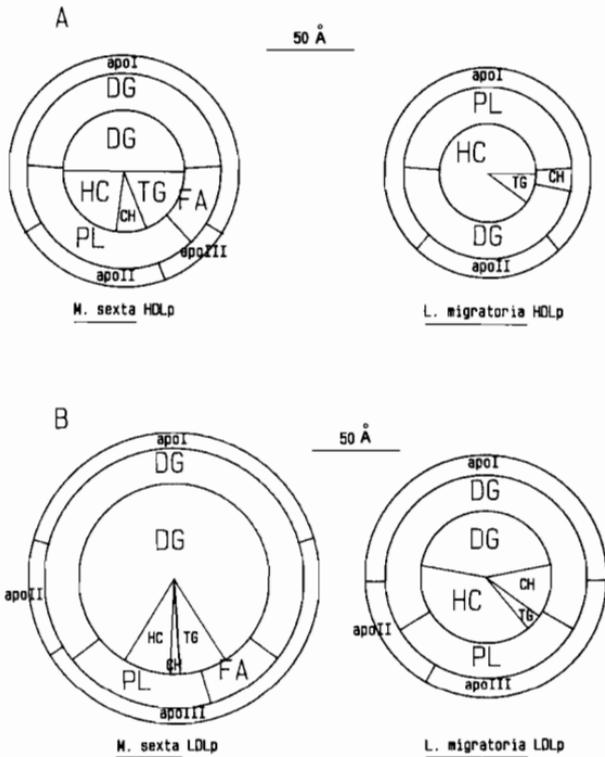


Figure 2 Schematic representation of the structure of HDLp (A) and LDLp (B) from *Manduca sexta* and *Locusta migratoria*. The figure shows the composition of the lipid core and surface monolayer as well as the proportion of various apoproteins. Based on data from References 9, 15, 65, 76. CH, cholesterol; DG, diacylglycerol; FA, free fatty acid; HC, hydrocarbon; PL, phospholipid; PR, protein; TG, triacylglycerol.

Figure 2 also shows the relative proportion of apoproteins in the lipoproteins, but the model should not be taken literally with regard to the actual location of the apoproteins in lipoprotein. A few studies have addressed the question of location directly. In the larval HDLp from *M. sexta*, apoLp-I appears to coat the surface, since it is readily iodinated and cleaved by trypsin, and antibodies raised against the intact lipoprotein react with apoLp-I (55, 61, 78). On the other hand, apoLp-II is somehow sequestered in the lipoprotein, since it is resistant to trypsin treatment or iodination, and antibodies raised against apoLp-II do not react against intact lipoprotein (55, 61, 78). Similar conclusions were reached for apoLp-I and -II of the locust and cockroach (42). Monoclonal antibodies raised against locust LDLp showed

specificity for apoLp-I, -II, and -III (77a). In accord with results from studies using polyclonal antibodies, it was found that apoLp-II is less exposed than apoLp-I in both LDLp and HDLp (77a). The structures of the asparagine-linked oligosaccharides of locust apoproteins have been determined (58) and are of the high-mannose type.

Based on circular dichroic and infrared spectra, Kashiwazaki & Ikai (42) have proposed that the apoproteins of locust and cockroach lipophorin are predominately present as extended β -sheets, as seems to be the case for apo-B in mammalian LDL (13). This suggestion is consistent with all the known properties of apoLp-I. However, since apoLp-I accounts for about 75% of the mass of the apoproteins in lipophorin and would dominate the spectral properties of the intact lipoprotein, it is not possible to draw firm conclusions about apoLp-II. Certainly the properties of apoLp-II suggest that it is buried in the lipoprotein, but cross-linking studies place it in close proximity to apoLp-I (42).

LIPOPHORIN METABOLISM DURING FLIGHT

The most thoroughly studied aspect of lipophorin function is its role in flight metabolism. For background, the reader is referred to a recent review (2). In locusts and Lepidoptera, the fat body is the source of lipid, which fuels their flight. Mobilization of lipid from the fat body is effected by adipokinetic hormone, which causes the triacylglycerol stores to be converted to diacylglycerol and released from the fat body (29, 92-98, 105, 106, 109, 112). The mechanism of diacylglycerol release is not understood at present, but it clearly does not involve the biosynthesis of new apolipoproteins or new molecules of lipophorin (63). Although experimental data are meager at present, it seems reasonable to propose that diacylglycerol accumulates in the plasma membrane of the fat body cells and then moves via fluid-phase diffusion into HDLp. The lipid transfer protein described by Ryan et al (76, 77) may have a role in this transfer process.

Regardless of the actual transfer mechanism, diacylglycerol is added to HDLp, and the particle begins to expand as this occurs. The capacity for HDLp to accept diacylglycerol would be limited, since the expansion of the core volume would expose lipid to water, were it not for the presence of apolipophorin-III in the hemolymph. ApoLp-III is a small (18-20 kd) apoprotein that has high affinity for lipid-water interfaces (49). Thus apoLp-III binds to the newly created lipid-water interface and stabilizes the expanding particle. In *M. sexta* the fully loaded lipoprotein (LDLp) has nearly doubled its molecular mass, with diacylglycerol accounting for 70% of the increase and apoLp-III the remainder (21). In *L. migratoria* LDLp increases its mass by about 50%, with equal contributions from diacylglycerol and apoLp-III (9).

ApoLp III has been purified from *M. sexta* (48, 104), *Thasus acutangulus*, the mesquite bug (104), *Gastrimargus africanus* (37), and *L. migratoria* (20). In the last two insects the protein is glycosylated, whereas in the first two it is not. The protein from *M. sexta* has been the most extensively studied and its amino acid sequence (Figure 3) has been determined from cDNA and protein sequencing (21). The cDNA sequence suggests the presence of a prepro peptide. The protein seems to be composed of repeating tetradecapeptide units, each of which has the potential to form amphiphilic helices.

Physical studies of *M. sexta* apoLp-III have shown that it has a high affinity for both phospholipid and diacylglycerol surfaces. It is highly asymmetric for such a small protein and seems to be a prolate ellipsoid with an axial ratio of 3. When spread on the air-water interface, the protein exhibits the unusual ability to form a stable monolayer at very high surface pressures. At the interface it exists in two states: an expanded state in which the molecule appears to be unfolded and a compressed state in which the molecule has the same shape as found in solution (49). In the latter state the protein seems to be attached to the interface by only a fraction of its surface, perhaps end on (Figure 4).

Wells et al (103) have shown that during the AKH-induced loading of lipid into HDLp, a lipoprotein that is intermediate in density between HDLp and LDLp is formed. Based on the properties of both the intermediate and apoLp-III, the authors proposed that apoLp-III binds to the surface of the

Met	Ala	Ala	Lys	Phe	Val	Val	Val	Leu	Ala	Ala	Cys	Val	Ala	Leu	Ser	His	Ser	Ala	Met
			-20					-15					-10					-5	
Val	Arg	Arg	ASP	ALA	PRO	ALA	GLY	GLY	ASN	ALA	PHE	GLU	GLU	MET	GLU	LYS	HIS	ALA	LYS
		-1	1				5					10					15		
GLU	PHE	GLN	LYS	THR	PHE	SER	GLU	GLN	PHE	ASN	SER	LEU	VAL	ASN	SER	LYS	ASN	THR	GLN
		20					25					30					35		
ASP	PHE	ASN	LYS	ALA	LEU	LYS	ASP	GLY	SER	ASP	SER	VAL	LEU	GLN	GLN	LEU	SER	ALA	PHE
		40					45					50					55		
SER	SER	SER	LEU	GLN	GLY	ALA	ILE	SER	ASP	ALA	ASN	GLY	LYS	ALA	LYS	GLU	ALA	LEU	GLU
		60					65					70					75		
GLN	ALA	ARG	GLN	ASN	VAL	GLU	LYS	THR	ALA	GLU	GLU	LEU	ARG	LYS	ALA	HIS	PRO	ASP	VAL
		80					85					90					95		
GLU	LYS	GLU	ALA	ASN	ALA	PHE	LYS	ASP	LYS	LEU	GLN	ALA	ALA	VAL	GLN	THR	THR	VAL	GLN
		100					105					110					115		
GLU	SER	GLN	LYS	LEU	ALA	LYS	GLU	VAL	ALA	SER	ASN	MET	GLU	GLU	THR	ASN	LYS	LYS	LEU
		120					125					130					135		
ALA	PRO	LYS	ILE	LYS	GLN	ALA	TYR	ASP	ASP	PHE	VAL	LYS	HIS	ALA	GLU	GLU	VAL	GLN	LYS
		140					145					150					155		
LYS	LEU	HIS	GLU	ALA	ALA	THR	LYS	GLN											
		160					165												

Figure 3 Amino acid sequence of *Manduca sexta* apolipoprotein-III (21). Residues in lower case with negative numbers refer to the signal peptide deduced from cDNA sequencing. Upper-case residues with positive numbers refer to the sequence of the mature hemolymph protein.

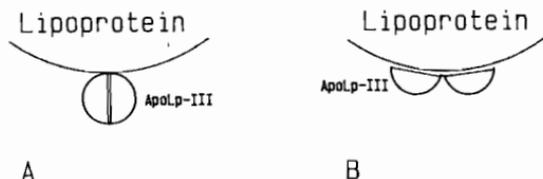


Figure 4 Hypothetical model of the two conformations of apolipoprotein-III found during formation of LDLp (49, 103). ApoLp-III is proposed to bind to the intermediate lipoprotein with its minor axis parallel to the surface (A) and to unfold on the surface as more diacylglycerol is added to the lipoprotein (B).

intermediate in a manner similar to that described above for its compressed state at the air-water interface. As further diacylglycerol is added to the particle, apoLp-III undergoes a conformational change, unfolding on the surface and thereby covering a larger area of lipid-water interface (see Figure 4). Data in the literature suggest that an intermediate is also produced during the formation of LDLp in *L. migratoria*, although the data were not originally interpreted in this manner (106).

Although preliminary data show little sequence identity among apoLp-IIIs from different insects, the surface properties of the proteins might be more critical than the sequence for the role of stabilizing the expanding surface created during diacylglycerol uptake. Supporting this view, Van der Horst et al (D. J. Van der Horst, R. O. Ryan, M. C. Van Heusden, T. K. F. Schulz, J. M. Van Doorn, et al, unpublished information) have shown that the conversion of HDLp to LDLp by an in vitro fat body preparation from *L. migratoria* is supported equally well whether *L. migratoria* or *M. sexta* apoLp-III is added to the system.

The mechanism of delivery of diacylglycerol from LDLp to flight muscle is not well understood at present. There is evidence of a membrane-bound lipase in *L. migratoria* flight muscle that has a preference for LDLp over HDLp (99, 108, 110). Whether apoLp-III has any role in this specificity is not established (107), although LDLps containing either *L. migratoria* or *M. sexta* apoLp-III deliver diacylglycerol to *L. migratoria* flight muscle equally well (D. J. Van der Horst, R. O. Ryan, M. C. Van Heusden, T. K. F. Schulz, J. M. Van Doorn, et al, unpublished information).

Last instar larvae of *M. sexta* and *L. migratoria* show a minimal capacity to produce LDLp in response to AKH (75, 92). This limitation is not the result of the low hemolymph levels of apoLp-III found in larvae, since increasing its concentration by injecting apoLp-III did not augment the AKH response (75, 92). It has been suggested that this lack of response to AKH results from the absence of an AKH-dependent lipase in larval fat body (92).

VERY-HIGH-DENSITY LIPOPROTEINS

While lipophorin in various forms from very high to low density seems to be a hemolymph component in all insects, a number of relatively lipid-poor, very-high-density lipoproteins have also been isolated from insect hemolymph and characterized. Isolation by ultracentrifugation is relatively easy (38). Some of these lipoproteins are found only in one life stage or are sex specific, e.g. vitellogenins. These VHDL particles cannot be placed into a single structural category. It appears that different patterns have developed to meet different needs.

The best characterized VHDLs are vitellogenins and vitellins. Good reviews of earlier work on these proteins are available (2, 27, 31, 51). It is not clear that all vitellogenins and vitellins are lipoproteins; those of *Drosophila* appear not to be. However, in most other species that have been analyzed, these proteins contain about 10% lipids, which generally consist of a mixture of phospholipids, glycerides, sterols, and hydrocarbons in proportions not unlike those found in lipophorins. Indeed, it is generally accepted that one of the functions of vitellogenin is the transport of lipids from the fat body, where vitellogenin is synthesized and assembled, to the oocyte, where lipids serve as energy stores in embryogenesis and early larval life.

A major problem in studying the structural organization of vitellogenins and vitellins is their extreme sensitivity to proteolysis. This is especially true with vitellins, which must be isolated from egg homogenates, which are especially rich in proteases (32). Without extreme care and the use of a battery of protease inhibitors, it is unlikely that the isolated product will truly reflect the nature of the material in situ in the oocyte. Even when proteolysis is extreme, the lipoproteins can migrate as single bands in native polyacrylamide gels, as the peptides are held together by hydrophobic effects. We give two examples to illustrate this problem. Lensky & Skolnik (53) reported that *Apis mellifera* vitellogenin consisted of 26 peptides, as determined by SDS polyacrylamide gel electrophoresis; Harnish & White (32), using protease inhibitors, showed that honey bee vitellogenin and vitellin consist of a single 180-kd polypeptide. A recent paper by Borovsky & Whitney (5) has suggested that vitellin of *Aedes aegypti* consists of six polypeptides, while papers by three other groups (30, 54, 68) had reported only two polypeptides in vitellin or vitellogenin of the same species. We have also reported difficulties with the vitellogenin of *M. sexta* (39, 60), which contains two apoproteins, apovitellogenin I and II (apoVg-I and -II). The large apoprotein is extremely sensitive to proteolysis, and only by examining the vitellogenin from the fat body can one see that apoVg-I consists of a single polypeptide of 177 kd. Earlier workers often felt that the insidious effect of proteolysis could be avoided by adding inhibitor phenylmethane sulfonyl fluoride (PMSF) to

homogenates. Unfortunately, this compound is not very efficient and can act only against one class of proteases, the serine proteases. In order to obtain truly native vitellins, it will probably be necessary to use a battery of different protease inhibitors (66). Even the important comparative study of Harnish & White (32) used only inhibitors against serine proteases, and some of their findings may need to be reinvestigated.

Taking the reported results (32, 51, 112) on face value, it appears that there are several classes of vitellogenins, based upon the number of polypeptide chains and the size of the intact lipoprotein. Perhaps analysis of the gene structure and of the transcribed messages will allow a better understanding of the relationships among vitellogenins. It has been shown that all of the polypeptides of locust vitellogenins are derived from two primary translation products, probably one from each vitellogenin gene (112). Wyatt et al (112) have argued that other vitellogenins (excluding those of *Drosophila*) may be similarly derived. It is worth noting that vitellogenins of Lepidoptera and mosquitoes are somewhat analogous to lipophorins, containing large (180–200 kd) and small (40–70 kd) apoproteins, except that the vitellogenin contains two copies of each apoprotein and has a lower lipid content (8–12%). The particle size of these vitellogenins (~500 kd) is very similar to that of lipophorins. *Drosophila melanogaster* vitellogenin contains only small apoproteins (44–49 kd), while that of the honey bee contains only a large (180 kd) apoprotein. This suggests that portions of a primordial gene may have been lost in these species.

Insect eggs contain large amounts of vitellin and smaller amounts of other proteins, including lipophorin. Chino et al (8) have argued that because the insect egg contains larger amounts of lipids than can be accounted for by the amounts of vitellin (derived from hemolymph vitellogenin) and lipophorin, the latter must participate as a shuttle that carries lipid from the stores in the fat body to the ovary.

Another VHDL isolated from *M. sexta* hemolymph is a lipid transfer protein (LTP), which may participate in the transfer or exchange of lipid to and from lipophorin (77). LTP also contains a large apoprotein (~320 kd) and a medium-sized apoprotein (~85 kd), and is thus analogous to lipophorin and vitellogenin from the same species. Its lipid complement is similar to that of lipophorin and constitutes 10–15% of the total weight.

Hauerland & Bowers (34) reported a VHDL blue biliprotein from *Heliothis zea*. It contains biliverdin as a chromophore and appears to be composed of four identical apoproteins (150 kd each) and to contain 8.4% lipid. It is a glycoprotein of the (mannose)₉(*N*-acetylglucosamine)₂ type (N. H. Hauerland, unpublished information). The biliprotein appears in the hemolymph in the fifth instar and then is rapidly sequestered into the fat body, much like the larval storage proteins (N. H. Hauerland, unpublished in-

formation). Similar biliproteins have been identified in *Tricoplusia ni* and *Spodoptera* (G. Jones, R. O. Ryan, N. H. Haunerland, J. H. Law, unpublished information).

A VHDL of somewhat similar properties, but lacking a pigment, has been isolated from larval honey bees (81). The two apoproteins, each of 160 kd, are identical, as indicated by the single N-terminal sequence of amino acids. As with the *H. zea* biliprotein, rapid disappearance of the VHDL from the hemolymph at pupation suggests a role in metamorphosis. The role of these proteins in lipid transport is not clear at present.

The arylphorins (73), a class of larval serum proteins or storage proteins, are usually associated with small amounts (2–5%) of lipids. They selectively bind xenobiotics (35), and it has been suggested that they are carriers for ecdysteroids (26). Their role in lipid transport has yet to be defined. They may, like mammalian serum albumin, be nonspecific transport vehicles for hydrophobic material.

DIRECTIONS FOR FUTURE RESEARCH

This review clearly demonstrates the importance of lipid-transporting proteins. They have integral roles in all insect species and life stages, in numerous physiological processes such as growth, metamorphosis, reproduction, and flight. Much research on them remains to be done. In the case of lipophorin, nothing is known about intracellular assembly, the mechanism of lipid delivery, endocrine control of metabolism, or control of apolipoprotein gene expression. The control of vitellogenin gene expression and the sequestration of vitellogenin into the egg are exciting areas for future investigation.

To conclude, we mention yet another function of lipophorin that may prove fruitful for further research: defense of the insect against threatening organisms and toxins. The defense of insects against penetration by parasites, predators, and pathogens has been studied extensively, and such mechanisms of defense as melanotic encapsulation of microorganisms, lysis by bacteriostatic proteins, and phagocytosis have been described (e.g. see 69). Hemolymph coagulation has also been well studied as a defense against cuticular penetration. However, only recently has the role of lipophorin in coagulation been recognized (3). Close interaction between a type of hemocyte (the coagulocyte) and a soluble coagulogen (lipophorin) results in cross-linking of the lipophorins to form an insoluble clot. Little is known about the mechanism of clot formation, although free amino groups may have a role (4).

A second role of lipophorin in defense of the insect may lie in protection against toxins such as insecticides. The ability of hemolymph proteins to bind

insecticides was recognized in 1975, with the discovery that after topical application to the cockroach *Periplaneta americana*, DDT was bound to a hemolymph protein of >160 kd (59, 82). Further evidence defined the protein as a lipoprotein of approximately 520 kd (82, 111), with properties consistent with what is now known as lipophorin. An *in vivo* study finally demonstrated identity of the binding molecule with lipophorin in *M. sexta* (47). Haunerland & Bowers (35) showed that the proteins responsible for insecticide binding in *H. zea* include arylphorin and demonstrated partitioning of insecticides between arylphorin and lipophorin, based on the polarity of the xenobiotic.

The role of these hemolymph proteins in insect responses to toxins is not well defined, however. The proteins may increase adsorption of xenobiotics from cuticle or midgut, sequester xenobiotics or deliver them to detoxification sites, or deliver them directly to sites of intoxication. Thus, hemolymph proteins may confer protection on the insect by retarding absorption or hastening excretion, or may increase susceptibility of the insect by hastening delivery to sites of intoxication.

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